Advanced MRI in diabetes

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CEREBRAL BIOMARKERS OF COGNITIVE DECREMENTS

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Voor pap en mam

Contents

Chapter 1

General introduction

1

Diabetes mellitus is a common and chronic metabolic disease that affects the global population. In 2011, 366 million people worldwide suffered from diabetes.[1] The prevalence of diabetes is increasing rapidly with today's unhealthy lifestyle and increase in life expectancy, and is expected to rise to 552 million by 2030.[1] In the Netherlands, approximately 4.9% of the population was diagnosed with diabetes by a general practitioner in 2011.[2]

Diabetes is characterized by elevated blood glucose levels (i.e. hyperglycemia) caused by insulin deficiency and/or insulin resistance[3,4] and is often accompanied by obesity, hypertension, and dyslipidemia. Prolonged exposure to hyperglycemia leads to microvascular complications such as nephropathy, retinopathy and neuropathy, and macrovascular complications such as cardiovascular, cerebrovascular, and peripheral arterial disease.[5-7] Diabetes not only leads to damage in many organs of the body, but also affect the brain in terms of cognitive decrements, accelerated cognitive decline, and a twofold increased risk to develop dementia, including Alzheimer's disease.[8-10] All this causes a decrease of the quality of life, including less self care and less abilities to maintain social contacts.

Brain magnetic resonance imaging (MRI) studies have already demonstrated various structural abnormalities as atrophy, white matter lesions, and lacunar infarcts in patients with diabetes.[11] In addition, these brain abnormalities were related to cognitive impairment in diabetes.[12,13] However, the exact mechanisms underlying this cognitive decline still remain to be elucidated. Various mechanisms, including vascular, glucose-mediated, insulin-mediated, and inflammatory processes, have been proposed in this regard.[14-16] For example, diabetes is associated with atherosclerosis and an increased stroke risk, which indicates the role of vascular mechanisms. In addition, hyperglycemia might lead to abnormalities in cerebral capillaries[10] and might have a toxic effect, mediated by advanced glycation end-products (AGEs) and/or reactive oxygen species (ROS). These AGEs and ROS could lead to irreversible degeneration of functional and structural proteins, inflammation, or cerebral damage due to oxidative stress. Furthermore, altered insulin (and glucose) levels could also affect the amyloid metabolism, which explains the increased risk of diabetes to develop Alzheimer's disease.[10] Moreover, inflammatory processes are likely involved in cerebral alterations in patients with diabetes.[17,18]

As atrophy, whiter matter lesions, and lacunar infarcts reflect macrostructural end-stage effects, structural MRI is probably not sensitive enough to detect and unravel early cerebral changes associated with cognitive decline. Early cerebral changes may be reversible or could possibly be postponed with pharmacological intervention and this may have a beneficial effect on cognitive status and the quality of life. As an addition to structural imaging, more-advanced MRI techniques provide the opportunity to detect abnormalities at the microstructural, microvascular, metabolic, and functional level. However, studies into diabetes utilizing these MRI techniques are scarce. Therefore, this thesis aims to gain more insight into the neuronal mechanisms underlying cognitive

decrements in type 2 diabetes by applying these more-advanced and potentially more sensitive MRI techniques (Figure 1.1). Accordingly, first, some background will be provided regarding type 2 diabetes, the relation between type 2 diabetes, cognition, and MRI findings, pre-diabetes, the study design, and finally the outline of the thesis will be given.

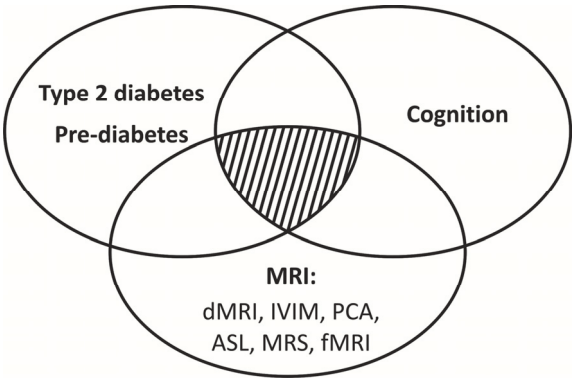

Figure 1.1 The main focus of this thesis is to elucidate the nature of the link between type 2 diabetes (and pre-diabetes), cognition, and findings by more-advanced magnetic resonance neuroimaging techniques (shaded area), to gain more insight into the underlying neuronal mechanisms of cognitive decrements in type 2 diabetes. dMRI, diffusion MRI; IVIM, intravoxel incoherent motion MRI; PCA, phase-contrast MR angiography; ASL, arterial spin labeling; MRS, proton magnetic resonance spectroscopy; fMRI, functional MRI.

Diabetes mellitus

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia due to insulin deficiency and/or insulin resistance[3], and is subdivided into a type 1 diabetes and type 2 diabetes group. Approximately 10% of all the patients with diabetes are diagnosed with type 1 diabetes mellitus[2], which commonly occurs in childhood or adolescence as a result of absolute insulin deficiency. The majority (approximately 90%) of patients with diabetes is diagnosed with type 2 diabetes mellitus[2] due to insulin resistance and relative insulin deficiency. Insulin resistance is largely driven by obesity, physical inactivity, and an unhealthy (i.e. high fat) diet and therefore type 2 diabetes is often associated with hypertension, obesity, and dyslipidemia. Eventually, the reduced sensitivity to insulin will lead to elevated blood glucose levels on which the diagnosis is established. This thesis mainly focuses on cerebral MRI findings in participants with type 2 diabetes, who are considered to have diabetes according to the World Health Organization 2006 criteria.

Type 2 diabetes and cognition

A detailed overview of this topic, combined with MRI findings, will be given in chapter 2. As already mentioned, type 2 diabetes is associated with cognitive decrements[19], accelerated cognitive decline[8], and an increased risk for developing dementia and Alzheimer's disease.[8,10,14,20,21] Two cognitive domains that are most consistently and relatively early affected in type 2 diabetes are memory and processing speed.[22-24] One region of the brain that plays an essential role in memory is, for instance, the hippocampus, while processing speed is more attributed to multiple brain regions globally distributed throughout the entire brain. The effects of cognitive decrements in type 2 diabetes are mild and evolve slowly over time, but will intensify in older patients.[25] Till today, the development of cognitive decrements in type 2 diabetes is not well understood.

Type 2 diabetes and MRI

Atrophy, white matter lesions, lacunar and cortical infarcts are common clinical findings in patients with type 2 diabetes.[11,26,27] A detailed overview of brain MRI abnormalities in type 2 diabetes is also given in chapter 2. It is interesting to identify whether functional and/or physiological cerebral characteristics precede these cerebral abnormalities. In this context, the use of advanced MR neuroimaging techniques, such as diffusion MRI (dMRI), intravoxel incoherent motion (IVIM) MRI, arterial spin labeling (ASL), MR spectroscopy (MRS), and functional MRI (fMRI), could be relevant to identify early MRI biomarkers that precede cognitive problems. In brief, dMRI will provide measures of the structural organization of the white matter, IVIM will examine the microvasculature and microstructure of the brain, ASL provides more insight into cerebral blood perfusion, MRS offers a window on cell metabolism, and fMRI measures the hemodynamic (blood oxygenation) response related to neuronal activity.

Until 2011 (i.e. the start of the work described in this thesis), the number of type 2 diabetes studies including these more-advanced techniques was limited. Only one fMRI study was published and the authors observed reduced functional connectivity in patients with type 2 diabetes.[28] Two dMRI studies reported on microstructural abnormalities in various brain regions in adolescents and older patients with type 2 diabetes.[29,30] Another two studies applied MRS in patients with type 2 diabetes.[31,32] Only one study observed different metabolite levels (i.e. higher myo-inositol ratios) in type 2 diabetes[31], while the other study did not find any altered metabolic levels (i.e. *n*-acetyl aspartate and choline ratios).[32] Both studies did not observe any relationship between metabolite levels and cognition. In this thesis, we apply a number of different more-advanced MRI techniques to the same diabetes population.

Therefore, the central research question to be addressed in this thesis is:
Can more-advanced MR neuroimaging techniques give us additional insight into the neuronal mechanisms underlying cognitive decrements in type 2 diabetes?

Pre-diabetes

The progression of normal glucose metabolism to type 2 diabetes is a gradual process in which insulin resistance plays a crucial role. Insulin resistance, before the clinical presentation of type 2 diabetes, is often accompanied by other metabolic and vascular abnormalities. The cluster of these cardiovascular risk factors is referred to as the metabolic syndrome and can be considered as a pre-diabetic condition.[33] Interestingly, patients with the metabolic syndrome have a high likelihood to develop type 2 diabetes and may display similar cognitive decrements as seen in patients with type 2 diabetes.[34] Furthermore, the cardiovascular risk factors are associated with an increased risk of late-life dementia.[35] From a healthcare perspective it is therefore highly relevant to identify early MRI cerebral abnormalities related to the subtle cognitive decrements in the pre-diabetic group.

Study designs

In this thesis, two different study designs were used to gain more insight into the mechanisms underlying cognitive decrements in type 2 diabetes. In design 1, The Maastricht Study data were used to recruit participants for additional brain MRI measurements. In design 2, the neuroimaging data were used of The Maastricht Study (described below). The selection procedure of participants in design 1 was based on cognition, while the selection in design 2 was based on (pre-)diabetes status (Figure 1.2).

The Maastricht Study is an ongoing observational, prospective, population-based cohort study that focuses on the etiology, pathophysiology, complications, and comorbidities of type 2 diabetes and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands.[36] The participants underwent an extensive battery of measurements, including blood sampling, blood pressure measurements, and assessment of cognitive performance, for example. The baseline survey examinations of each participant were performed within a time window of three months.

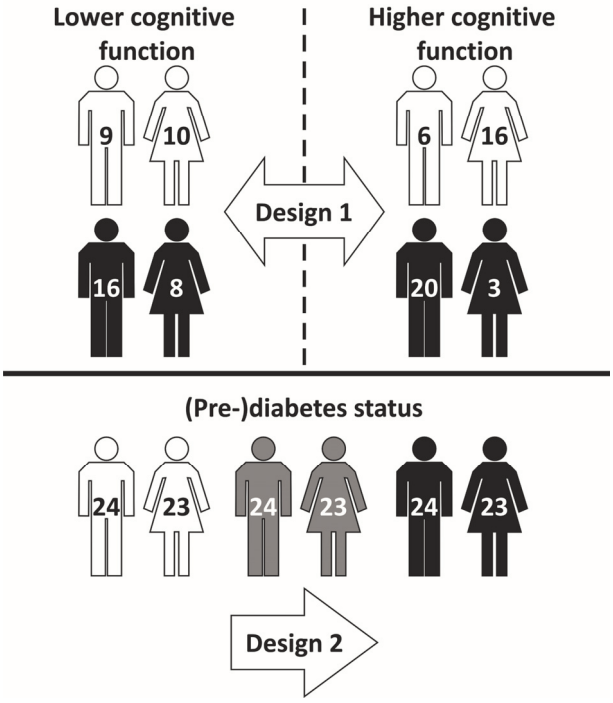

Figure 1.2 Schematic overview of the included participants in the two study designs used in this thesis. In design 1, the selection of participants was based on cognition subdivided into a lower and a higher cognitive function group. In design 2, the participants were selected based on (pre-) diabetes status. White, gray, and black male/female represent the totally included participants with neither type 2 diabetes nor pre-diabetes (controls), participants with pre-diabetes, and participants with type 2 diabetes, respectively.

Selection based on cognition

The research described in chapters 3 through 6 of this thesis is based on a selection of the first 866 Maastricht Study participants, who completed the baseline survey examinations between November 2010 and March 2012. A broad range of cognitive performance was selected to increase the probability of finding MRI differences associated with cognitive decrements. Participants, who fulfilled the selection criteria, were invited to participate in this additional MRI study. A total of 43 participants with a lower and 45 participants with a higher cognitive performance score, matched on age, sex, and education level, were included in this MRI study (Panel 1.1). In addition, participants with type 2 diabetes were equally divided over both groups (Figure 1.2; design 1). A more detailed selection procedure is provided in the Supplementary Data of chapter 4.

1

Selection based on (pre-)diabetes status

The research described in chapter 7 of this thesis is based on a selection of age, sex and education matched participants (pre-diabetes, type 2 diabetes, and participants with neither type 2 diabetes nor pre-diabetes) from the fMRI data of The Maastricht Study (Panel 1.2). A total of 47 matched participants for each group were included in the MRI study of chapter 7 (Figure 1.2; design 2).

Panel 1.1: Selection based on cognition (Design 1)

The subdivision of participants in two groups was based on a cumulative score of three neuropsychological tests: *1*) 15-Word Learning Test[37], which covers the domain of verbal memory, *2*) the Stroop Color-Word Test[38], which covers the domains of attention and flexibility, and executive functioning, and *3*) Verbal Fluency Test[39], which covers a variety of domains including memory, processing speed, attention, and executive functioning. For matching, the scores of each neuropsychological test were adjusted for age, sex, and educational level. The cumulative score was calculated by adding the adjusted *z* scores of the three tests. To increase the probability of finding MRI differences related to cognitive performance, we, initially, only selected participants with the 30% lowest and 30% highest cumulative score.

Participants were excluded if *i*) the time span between enrollment in The Maastricht Study and MRI was >1.5 years, *ii*) they had an incomplete cognitive assessment, *iii*) a known history of or stroke or neurological disease such as Alzheimer's or Parkinson's disease, *iv*) type 1 diabetes, *v*) mild cognitive impairment or dementia, *vi*) metabolic syndrome, *vii*) color blindness, *viii*) unknown diabetes status, *ix*) an impaired fasting blood glucose level in participants without type 2 diabetes, and *x*) MRI contraindications. A total of 39 participants with the lowest and 34 participants with the highest cumulative score were enrolled in the MRI study.

To increase the total number of participants and to achieve a continuous scale in cognition scores, we additionally included 15 participants. These participants, who belonged to the average cumulative score (i.e. not to the 30% lowest and 30% highest cumulative score), were subdivided, together with the other 73 participants, to form a lower and a higher cognitive group. Finally, a total of 43 participants with a lower and 45 participants with a higher cognitive performance score, matched on age, sex, and education level, were included in this MRI study.

Panel 1.2: Selection based on (pre-)diabetes status (Design 2)

Data of the first 3451 participants who completed the baseline survey between November 2010 and September 2013 and from which 1168 participants were included in the MRI visit of The Maastricht Study between December 2013 and October 2014 were used for the selection of the participants. As MRI measurements were not performed in 59 participants, all (pre-diabetic) participants with both an impaired glucose tolerance and the metabolic syndrome were selected from the remaining 1109 participants. This resulted in a total of 47 participants with pre-diabetes. Next, an equal number of participants matched on age, sex, and educational level were selected from the type 2 diabetes group and from the group with neither type 2 diabetes nor pre-diabetes. In addition, no incidental neuroimaging findings were reported in the selected participants.

Outline of thesis

The overall aim of this thesis is to gain more insight into the neuronal mechanisms underlying cognitive decrements in type 2 diabetes by applying more-advanced and potentially more sensitive MR neuroimaging techniques. In other words, can we find early MRI abnormalities in the brain that are related to type 2 diabetes and cognitive decrements? Therefore, in this thesis, a broad spectrum of different and noninvasive MRI techniques is investigated. The research described in chapter 3 to 6 includes the selection of participants based on cognition, while the research described in chapter 7 was based on (pre-)diabetes status.

Chapter 2 provides a narrative review of the literature on various (potentially) pathological brain abnormalities associated with type 2 diabetes and cognitive decrements. Addressed are the various types of cerebral abnormalities and appropriate neuroimaging techniques available to study the pathophysiology in the range from routine clinical application to explorative research.

In **chapter 3**, dMRI is used and constrained spherical deconvolution based fiber tractography is applied to examine whether the white matter connectivity of the hippocampus to other brain regions is affected and related to the memory decrements in participants with type 2 diabetes.

In **chapter 4**, IVIM, which is another MRI technique based on diffusion, is performed to simultaneously examine the local microstructural and microvascular properties of the hippocampus in participants with type 2 diabetes. In addition, the relation between these hippocampal properties and memory performance are examined.

In **chapter 5**, a phase-contrast velocity-sensitive MR angiography and ASL MRI techniques are applied to investigate whether the blood supply from the common carotid artery to the brain and whether whole brain perfusion are altered in type 2 diabetes. In addition, the effects of cognitive performance on the perfusion measures are examined.

In **chapter 6**, proton MRS is conducted to assess whether neurotransmitters, i.e. the inhibitory γ-aminobutyric acid (GABA) and the excitatory glutamate, are related to type 2 diabetes and cognition.

In **chapter 7**, resting state fMRI is used and graph theoretical network analysis is applied to obtain functional connectivity measures to examine global alterations in functional networks in participants with type 2 diabetes, pre-diabetes, and healthy controls. In addition, the relation between the obtained graph measures and information processing speed is investigated.

In **chapter 8**, all the key findings of the above mentioned chapters are combined and discussed. In addition, clinical implication, methodological considerations, and directions for future research are addressed.

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Chapter 2

Cerebral pathology and cognition in diabetes: The merits of multiparametric neuroimaging

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Submitted for publication

Abstract

Type 2 diabetes mellitus is associated with accelerated cognitive decline and various cerebral abnormalities visible on MRI. The exact pathophysiological mechanisms underlying cognitive dysfunction in diabetes still remain to be elucidated. In addition to conventional (macrostructural) images, MRI offers a versatile set of novel contrasts and parameters (i.e. measures), related to various cerebral characteristics such as blood perfusion, neuronal function, white matter microstructure, and metabolic function. This review summarizes to what extent MRI, especially advanced multiparametric techniques, can elucidate the underlying neuronal substrate that reflect the cognitive decline in type 2 diabetes. More-advanced multiparametric MRI contrasts and the pertaining parameters are able to reveal abnormalities in perfusion, neuronal function, and white matter microstructure in type 2 diabetes, which may be related to cognitive decline. The reviewed studies point at multifactorial pathophysiological processes that underlie the cognitive impairment, including insulin, glucose, vascular, and inflammation related mechanisms. To further elucidate the nature and timely evolution of the link between diabetes, cognitive decline, and brain abnormalities, biomarkers are needed which can to some extent be provided by advanced, quantitative multiparametric MRI techniques.

Introduction

Type 2 diabetes mellitus is a common metabolic disorder, characterized by chronic hyperglycemia, in a context of insulin resistance and relative insulin deficiency.[1,2] Type 2 diabetes has commonly been considered a disease of elderly populations. However, with today's unhealthy lifestyle, also an increasing number of younger (that is, middle-age) people are developing diabetes.

Type 2 diabetes has a broad range of serious clinical complications, including nephropathy, retinopathy, and cardiovascular disease, and is often accompanied by cardiovascular risk factors such as hypertension and dyslipidemia. Type 2 diabetes is also associated with cognitive deficits, accelerated cognitive decline, an increased risk of dementia, and Alzheimer's disease (AD).[3-5] In type 2 diabetes, the rate of age-related cognitive decline due to neurodegenerative changes is increased[6], and cognitive changes mainly affect learning, memory and information processing speed.[5]

Unfortunately, the exact neuronal mechanisms underlying the cognitive decline associated with type 2 diabetes still remain to be elucidated. It has been suggested that various mechanisms – including vascular, insulin-mediated, glucose-mediated, and inflammatory processes – are relevant.[6-9]

In recent years, numerous studies have highlighted the adverse effects of diabetes on brain physiology and cognitive function to assess contributing pathophysiological mechanisms.[10,11] Most studies have applied conventional MRI with multiple contrasts to detect macrostructural cerebral changes. However, structural abnormalities on MRI reflect end-stage effects of impaired tissue, and is probably not sensitive enough to detect the earliest cerebral changes, expectedly more closely reflecting mechanisms, associated with cognitive decline.[12] For this purpose, more-advanced and potentially more-sensitive multiparametric MRI techniques, such as functional MRI (fMRI) and diffusion MRI (dMRI), can be used, which could lead to a better insight into the mechanisms that precede macrostructural (end-stage) abnormalities.

Key points:

- Multiparametric MRI techniques can provide novel insights into the nature of brain pathology and associated cognitive decrements.
- Cognitive pathology in diabetes is reflected not only by macrostructural cerebral alterations on MRI, but also by abnormalities in parenchymal and vascular microstructure, microvascular blood flow, and metabolism.
- More-advanced quantitative MRI techniques detect more subtle abnormalities than conventional MRI.
- Most alterations are found in frontal and/or temporal lobes, consistent with type of cognitive decline.
- For functional and diffusion based MRI techniques, especially cerebral network-based analyses are promising.

The present narrative review summarizes recent literature and provides an overview of the various brain abnormalities associated with type 2 diabetes in combination with cognitive decrements. It will explore the appropriate MRI techniques to study associations with cognitive performance in patients with type 2 diabetes (for an overview of typical abnormalities and the corresponding techniques, see Figure 2.1), will discuss the results and limitations of studies using neuroimaging techniques, and will make recommendations for future research from an imaging perspective. This review is structured according to the various types of cerebral abnormalities and the appropriate MRI techniques available to study pathophysiology, in the range from routine clinical application to explorative research.

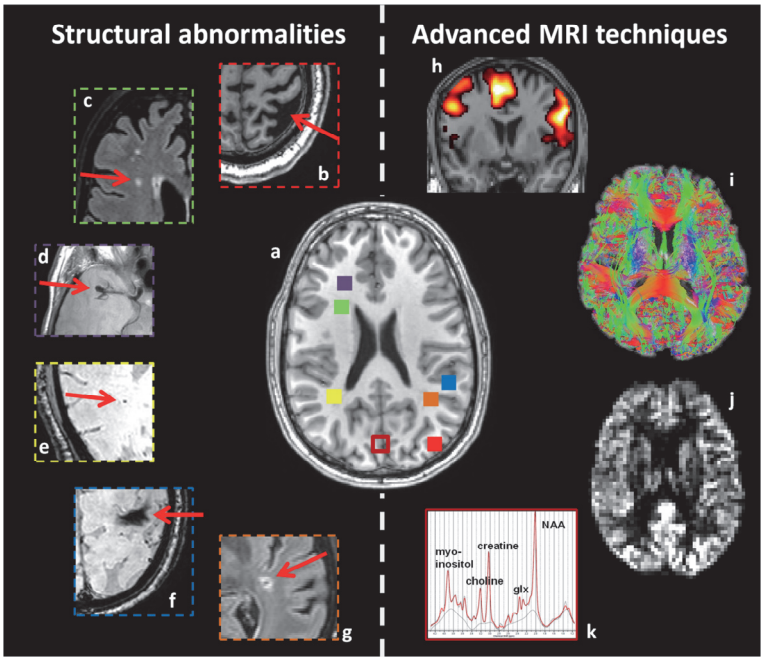

Figure 2.1 Overview of structural abnormalities which may be found in patients with type 2 diabetes (**b-g**), and advanced MRI techniques sensitive to more subtle cerebral alterations (**h-k**). This figure is an illustration from the authors' clinic. (**a**) T1-weighted image of a healthy young brain. Structural abnormalities in patients with type 2 diabetes: (**b**) Atrophy (T1-weighted), (**c**) white matter lesions (FLAIR), (**d**) aneurysm (T2-weighted), (**e**) microbleeding (T2*-weighted), (**f**) macrobleeding (T2*-weighted), and (**g**) lacunar infarct (FLAIR). Advanced MRI techniques: (**h**) functional MRI, (**i**) diffusion MRI, (**j**) arterial spin labeling, and (**k**) magnetic resonance spectroscopy. Corresponding colored squares in (**a**) represent the approximate location where the structural abnormalities were found and where the single voxel for spectroscopy was located, respectively.

Atrophy

Cerebral atrophy can generally be defined as the shrinkage of brain tissue, which is a result of neurodegenerative processes, such as the loss of neurons and their interconnections.[13] Cerebral atrophy can be assessed using multiple MRI contrasts: T1-weighted, T2-weighted, fluid attenuated inversion recovery (FLAIR) and other contrasts.[14] In general, to assess atrophy reliably, excellent contrast between different tissue types (white matter, gray matter, and CSF) is required, ideally with high spatial isotropic resolution. For this purpose, it is advised to use high resolution (1.0-1.5 mm cubic voxels) preferably three-dimensional (thin section) T1-weigthed sequences, that are widely available.[15] Most T2-weighted scans are less appropriate due to thicker slices, but recently isotropic T2-weigthed scans are now also available on most modern scanners, preferably with 3.0T field strength and less cable signal losses by digitization of the coil signal. Furthermore, in addition to time consuming visual rating, (semi)automatic quantitative computational approaches are currently available with high reliability for a number of brain structures.

Many studies on type 2 diabetes, using various structural MRI techniques, report on atrophy.[14,16-21] Associations have been found between brain atrophy and decreased performance in various cognitive domains[22-25], including memory[16,17,22,26-36], attention and executive function[16,26,33-35,37-40], as well as processing speed[26,27,32-35], motor speed[30,41], and sensory speed.[32] Also progression of atrophy was found related to cognitive decrements in type 2 diabetes.[16,37,42,43]

Studies that investigated the underlying pathophysiological mechanisms in type 2 diabetes, showed that especially glucose- and insulin-mediated mechanisms are involved in cognitive decline, atrophy or progression of atrophy.[16,17,26,28,29,31,34,36,44] Moreover, one study reported that also inflammatory processes could play a role, because greater accumulation of tissue AGEs (advanced glycation end products) has been shown to partially mediate the association between gray matter atrophy and type 2 diabetes.[32]

Small vessel disease

Cerebral small vessel disease (cSVD) can be generally defined as pathological processes with various etiologies that affect the small arteries, arterioles, venules, and capillaries of the brain.[15] Signs of cSVD are white matter lesions, microbleeds, silent brain infarcts and lacunar abnormalities, which are also indicative for cognitive decline.[45]

White matter lesions

White matter lesions (WMLs) are typically observed as regions of bright, high-signal intensity in the white matter (i.e. white matter hyperintensities) depicted on T2-weighted and, especially, FLAIR images.[15] The underlying pathophysiology of WMLs is still poorly understood and is assumed to include multiple factors of vascular (through ischemia or arteriosclerosis) or inflammatory (through transudation of CSF) origin.[46] In WMLs, the white matter is affected, and as a result, the tissue is damaged, containing more water, and will have a somewhat longer T2 relaxation time. On a FLAIR T2-weigthed image, the bright signal from (pure) CSF is suppressed, allowing for a better visible contrast between normal appearing and affected (more aqueous) white matter. Therefore, although WMLs are also visible on T2-weighted scans, especially FLAIR scans are recommended for their detection.

WMLs are often divided in periventricular WMLs, which are located close to the ventricles, and deep WMLs, which are located in subcortical gray matter.[15] It was shown that periventricular, but not subcortical, WMLs are associated with the rate of cognitive decline in elderly non-demented participants.[47]

Numerous studies report on WMLs in patients with type 2 diabetes. More specific, deep (subcortical) WMLs[21,26,45,48], periventricular WMLs[27,45,49], and WMLs in general[27,35,44] are found in patients with type 2 diabetes. WMLs are also related with impaired cognition in type 2 diabetes[26,27,35,44,45,49,50], especially in the domains of processing speed[26,27], memory[27,35], attention and executive functioning[45,49], and motor speed.[45,49,50]

Only a few studies investigated possible underlying mechanisms involved in cognitive decline, and reported on the involvement of a vascular (i.e. blood pressure)[26,45], an inflammatory (i.e. periventricular WMLs associated with higher soluble intercellular adhesion molecule-1 (sICAM-1) levels)[49], or a glucose mechanism.[21,45,50]

Microbleeds

Cerebral microbleeds result from focal leakages of small blood vessels.[15] They are thought to be due to hemosiderin-laden macrophages in perivascular tissue, therefore containing iron deposits.[15] Compared with surrounding parenchyma, the iron causes a magnetic susceptibility difference, or magnetic field inhomogeneity and stronger T2* relaxation, both leading to signal intensity loss on T2*-weighted images. The detection of this signal loss (dark voids) is most apparent on T2*-weighted MRI sequences[15], and its sensitivity and size increases with magnetic field strength. Typically, microbleeds are found only incidentally on MRI, but are thought to play an important role in cognitive decline.[15] The reported prevalence of microbleeds increases with age[45] and is highly dependent on the used MRI sequence and field strength.

Microbleeds do not seem to be associated with type 2 diabetic patients with cognitive impairment[25], which remains the case at high field (7T).[51] Likewise, the evidence for the association of microbleeds with diabetes is also not strong.[35]

Silent brain infarcts

Silent brain infarcts (SBIs) are clinically asymptomatic (i.e. they lack stroke-like symptoms), but visible (generally 2-5 mm in diameter) as focal lesions on MRI, and are associated with cognitive deficits that commonly remain unnoticed.[52] These infarcts have high water content and therefore have roughly the same intensity as CSF and are defined as hyper-intense focal areas larger than 3 mm in diameter when detected on T2-weighted images, hypo-intense areas on T1-weighted images, and brighter areas on FLAIR images.[45,49,53] Small lesions located in the basal ganglia are often only visible on T2-weighted images, which are most sensitive for changes in water content, whereas lacunar infarcts in the white matter and brainstem can be distinguished from WMLs on T1-weighted images.[52]

Patients with type 2 diabetes often display SBIs, which are also related to impaired cognitive performance.[26,45,49,53] The number of SBIs and/or progression of SBIs are especially linked to decrements in motor speed, attention and executive functioning.[45,49,53] Possible underlying mechanisms involved in cognitive decline and SBIs are a vascular, a glucose-mediated, an insulin-mediated or inflammatory processes.[45,49,53] In addition, progression of SBIs is associated with higher sICAM-1 and high-sensitivity C-reactive protein (hs-CRP) levels, which indicates an important role of inflammatory processes.[49]

Lacunar abnormalities

Lacunes are pathologically defined as small areas (3-15 mm in diameter) of infarction, which is a result from an occlusion of one of the small penetrating branches of large cerebral arteries[15] and are associated with cognitive impairment.[54] In type 2 diabetes, lacunar infarcts often progress[19,55], which is likely caused by ischemia.[45] Lacunes are typically detected by using a combination of three scan sequences: FLAIR, T1-weighted, and T2-weighted images. The use of this combination of scan techniques is recommended as particular care is needed to differentiate lacunes from perivascular spaces.[15]

Cerebral infarcts (i.e. lacunar, cortical, subcortical infarcts, or infarcts in general) have been observed in patients with type 2 diabetes.[25,26,35,40,43,55] Most studies report a relationship between cerebral infarcts and decreased performance in various cognitive domains, including processing speed[26,35], sensory speed[55], memory[35,40], executive function[35,40], and global cognition.[40,43]

For the detection of cerebral atrophy or cSVD, various structural MRI techniques have been used. However, these techniques cannot unravel more subtle details of tissue alterations that underlie or precede the atrophy or cSVD. For this, more-advanced MRI techniques can be used, which will be discussed below.

Impaired cerebral perfusion

Cerebral perfusion is defined as the amount of blood flowing through a definite volume of tissue in a given time[56] and can be estimated using Arterial Spin Labeling (ASL) and Intravoxel Incoherent Motion (IVIM) imaging, or measured globally using a velocity-sensitive, phase-contrast MRI technique.[57-61] The ASL technique is based on magnetic labeling of arterial blood (e.g. blood in the common carotid artery), which is used as an temporary endogenous tracer for perfusion in the brain. The IVIM technique enables assessment of both the parenchyma and microvasculature and is based on the diffusion of water molecules in parenchyma and incoherent motion of water molecules in the microvasculature. The velocity-sensitive, phase-contrast MRI technique is based on differences in phase of the magnetic spins. An advantage of using ASL or IVIM is that these techniques make it possible to investigate regional differences related to disease pathology instead of only a gross measurement of total brain perfusion with phase-contrast MRI.[62,63]

A fair comparison between ASL and IVIM is not trivial due to the different complex physical mechanisms that contribute to the detected signal. However, the former is a truly quantitative method, which has been validated with PET perfusion measurements[64], whereas the latter is yet more experimental, though it can provide a higher signal-to-noise (SNR), and the possibility for an increased spatial resolution. Furthermore, ASL is still predominantly a gray matter technique, and not completely appropriate for areas with long arterial arrival times, low blood flow, such as white matter, or areas with collateral flow, whereas IVIM has no intrinsic limitations on arterial arrival time, allowing accurate measurement of perfusion throughout the entire vascular network including white matter.[65]

Thus far, only two studies, one using phase-contrast MRI (1.5T)[57] and one using ASL (3T)[60], did not find any differences in global perfusion between patients with type 2 diabetes and controls, while the other studies (ASL[59] and IVIM[61], both at 3T) observed regional differences in perfusion. Notably, these studies differ considerably regarding the applied MRI techniques, methodology, and magnetic field strength, which could explain these conflicting results.

One phase-contrast MRI study observed a positive association between perfusion and cognition, but this study was not able to explain the effect of diabetes on cognitive performance.[57] Another study did find a relation in patients with type 2 diabetes between perfusion and impaired cognition.[58] Promising results regarding reduced

cerebral perfusion in the insula cortex and cognitive performance in patients with type 2 diabetes were shown in a pilot ASL study.[59] After insulin administration, memory and verbal fluency improved, and perfusion was elevated in the (right) insula cortex of participants with diabetes, suggesting the involvement of an insulin mechanism. In type 2 diabetes, perfusion of the global gray matter was positively associated with verbal fluency[60], although local hippocampal perfusion (as measured using IVIM) had a negative association with memory performance.[61] These results suggest the involvement of a cerebrovascular mechanism, and that the association might be dependent on the brain region.

Taken together, all different perfusion techniques observed a relation with cognitive performance, which highlights the link between a vascular mechanism and cognitive decline. However, to observe regional (subtle) differences in perfusion, the more-advanced MRI techniques (i.e. ASL and IVIM) are better suited and contribute to important new knowledge to the understanding of cognitive decline in patients with type 2 diabetes. Additionally, higher field (>1.5T) MRI has been beneficial to elucidate differences in perfusion between patients with and without type 2 diabetes due to the increased sensitivity.

Neuronal dysfunction

Neuronal dysfunction refers to all impairments of the neuronal system, including reduced functional activity of certain brain regions and connectivity between different regions.[66] Functional MRI (fMRI) offers the opportunity to investigate to which extent neuronal regions are active, in terms of blood oxygenation changes. The underlying principle is that neuronal activity leads to locally increased blood flow and oxygenation. This changes the ratio of oxyhemoglobin to deoxyhemoglobin concentration, which can be detected on the basis of their differential magnetic susceptibility using T2*-weighted MR sequences, for which high field is beneficial due to an increase in intrinsic SNR, possible higher spatial resolution and importantly a stronger T2*-weighted contrast.

The amplitude of low frequency fluctuation (ALFF), a measure of spontaneous neuronal activity, regional homogeneity (ReHo), a measure of the neural regional synchronization, and functional connectivity, assessed by correlating time signals from distinct brain regions, were reported to identify abnormal brain activity in patients with type 2 diabetes.[66-69]

Reduced functional connectivity in the default mode network (DMN), i.e. the network of active brain regions when the brain is at (wakeful) rest and the participant is not focusing on anything particular, have been observed in patients with type 2 diabetes.[66,67,70-72] Reduced functional connectivity between the hippocampus and widespread regions in the DMN (Figure 2.2)[66], including the medial frontal cortex[71] has been reported, in addition to reduced functional connectivity between the posterior

cingulate and the medial frontal gyri and other regions in the DMN.[67,70] Reduced connectivity of the DMN was related to impaired memory[66,71], executive function[66], verbal fluency[71], and lower global cognition.[71] The disrupted functional connectivity in the DMN has been shown to be inversely correlated with insulin resistance[67] in type 2 diabetes, hinting at an underlying insulin-related mechanism. This thought is enhanced by the observation of acutely increased functional connectivity between the hippocampus and multiple regions in the DMN after intranasal insulin administration.[71]

ALFF and ReHo alterations have been reported in a variety of DMN brain regions (including temporal lobe and frontal lobes) in patients with type 2 diabetes.[68,69,73] The altered ALFF and ReHo values were related to impaired cognition, especially in the domains of attention and executive function[68,69,73], motor speed[68], processing speed[69], memory[69], and global cognition.[73] Moreover, ALFF values in the middle temporal gyrus were also inversely related to HbA_{1c}[68] and insulin resistance in the diabetic group was negatively correlated with altered neuronal activity.[69]

Altered brain activation has also been found in middle-aged patients with type 2 diabetes during a memory task, especially in task-related regions of the DMN[74], frontal cortex[74-76], and parietal cortex.[76] Moreover, activation in the task-related regions are positively associated with insulin resistance[74], HbA_{1c}[74,76], and plasma glucose[74] suggesting a major role of glucose metabolism.

Overall, all functional MRI studies consistently show evidence of altered neuronal activity or functional connectivity in combination with affected cognitive performance in patients with type 2 diabetes.

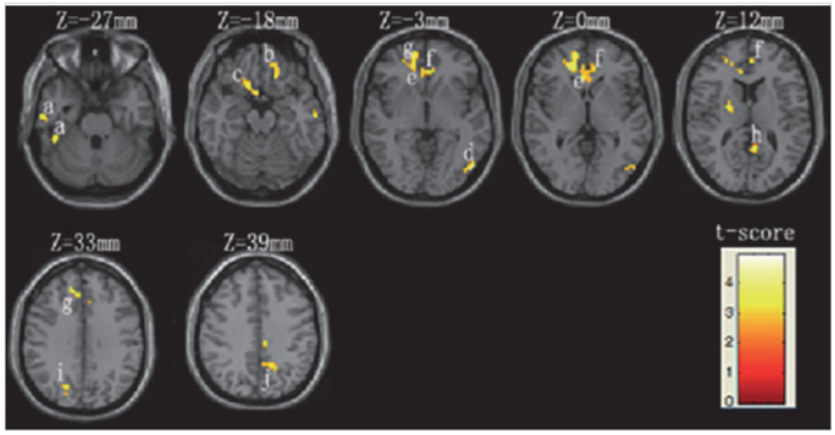

Figure 2.2 Regions of reduced functional connectivity with the left hippocampus in patients with type 2 diabetes and confirmed memory problems, compared with healthy controls: **a** = right Fusiform Gyrus, **b** = left Inferior Frontal Gyrus, **c** = right Inferior Frontal Gyrus, **d** = left Inferior Temporal Gyrus, **e** = right Anterior Cingulate Gyrus, **f** = left Anterior Cingulate Gyrus, **g** = right Medial Frontal Gyrus, **h** = left Posterior Cingulate Gyrus, **i** = right Precuneus, **j** = left Precuneus. Groups differences are overlaid on a spatially normalized T1-weighted MR image of an individual brain. Figure reprinted with permission from Zhou et al..[66]

White matter tract abnormalities

White matter tract abnormalities refer to impaired integrity or altered organization of axonal bundles and can be investigated using dMRI. This technique is based on diffusion of water molecules, and during the dMRI acquisition, tissue is sensitized with the local characteristics of molecular diffusion. The measures most often analyzed by dMRI are fractional anisotropy (FA) and mean diffusivity (MD). FA is a measure of tract directionality and MD is a measure of water diffusivity. Clinically, an increase in MD has been associated with reduced neuronal packing and increased extracellular space, possibly due to failure of neurogenesis or cell loss.[77] Recently, analysis methods have become available that allow the assessment of the integrity and efficiency of structural networks, using graph theoretical analysis on dMRI data.[78] For this, the acquisition of high quality dMRI data is recommended, including >30 diffusion sensitization directions, preferably at high field to benefit from optimal SNR characteristics. On the analysis side, to obtain widespread sets of tracts, it is recommended to apply highly sophisticated tractography methods such as probabilistic constrained spherical deconvolution[79], rather than the thus far most widely used clinical tractography method (diffusion tensor imaging).[80]

Microstructural abnormalities have been published for various brain regions in type 2 diabetes.[18,30,81,82] Reduced FA has been observed in the white matter[18,30] mostly concentrated in frontal and temporal regions[81], while elevated MD values were found in various brain regions, including the hippocampus[18] and multiple gray matter regions.[30] Temporal lobe abnormalities were associated with impaired memory.[61,81]

Altered network and structural connectivity in type 2 diabetes have been shown using tractography.[70,78,83] Local and global network properties (i.e. cluster coefficient, global efficiency, path length) were altered and associated with impaired processing speed.[78] Elevated MD and reduced FA were found in different tracts, including the superior longitudinal fasciculus[83], uncinate fasciculus[70,83], inferior longitudinal fasciculus[83], corpus callosum[83], and cingulum bundle.[70] These tract abnormalities were associated with impaired processing speed and memory[70,83] and highlight an underlying glucose-mediated mechanism as HbA_{1c} and fasting blood glucose were also related to these tract abnormalities.[70] In addition, reduced FA in the cingulum bundle correlated with lower functional connectivity between the medial frontal gyrus and the posterior cingulated cortex[70], suggesting that disruption in both functional and structural connectivity have one or more common underlying mechanisms involved in cognitive decline.

Diffusion MRI studies implicate that patients with type 2 diabetes do show evidence of white matter microstructure, tract, and network abnormalities.

Metabolic dysfunction

MR spectroscopy (^{1}H-MRS) enables the assessment of metabolic changes through the identification and quantification of spectral peaks associated with tissue metabolites.[84] ^{1}H-MRS is often used to investigate *n*-acetyl-aspartate (NAA), choline (Cho), creatine (Cr), myo-inositol (mI), γ-aminobutyric acid (GABA), and glutamate (Glu). NAA is a measure of neuronal integrity and a surrogate marker of normal functioning of neurons. Cho is an indirect marker of myelination and cell membrane metabolism. Cr is a measure of energy metabolism, and mI has been proposed as a glial marker and as an end-product of persistent hyperglycaemia.[84] GABA and Glu are major inhibitory and exhibitory neurotransmitters, respectively. However, detection and quantification of these neurotransmitter concentrations in vivo at low field strengths (<3T) are complicated due to spectral overlap with other metabolites. Recently, one study showed that Glu concentrations can be reliably estimated at 3T[85], which opens opportunities to study neurotransmitters in a clinical setting. As well for GABA, dedicated spectral editing sequences are available[86], but these techniques have not yet been applied to study type 2 diabetes. Another relevant metabolite in the context of diabetes is glucose, which typically requires high field strengths (>3T) for reliably detection with ^{1}H-MRS.[87] An alternative method to study brain glucose levels using MR spectroscopy is ^{13}C-MR spectroscopy, an approach that has been successfully applied to measure glucose metabolism in type 1 diabetes.[88]

MR spectroscopy studies on type 2 diabetes in relationship with cognition have been proven to be challenging, as no associations between metabolic alterations and cognitive performance were found.[89,90] An important remark is that these studies were performed at the rather low field strength (1.5T), which is likely not sensitive enough.

Interpretation

Table 2.1 provides an overview of all studies on type 2 diabetes, in which cognitive performance is related to diverse cerebral MRI contrasts. From this it can be appreciated that neuroradiologically visible MRI biomarkers (atrophy, WMLs, and lacunar abnormalities) and more subtle abnormalities (impaired cerebral perfusion, neuronal dysfunction, and white matter tract abnormalities) are related to cognitive decline, with a striking agreement between studies. For the other abnormalities (including microbleeds, SBIs, and metabolic dysfunction) the evidence of relationships with cognition is less convincing.

Most studies are associated with various methodological limitations. Most notably, typically only a limited number of subjects is included. Furthermore, the studies show a pronounced diversity regarding subject selection, matching of subjects, diagnosis and classification of diabetes, adjustment for risk factors, and data analysis methods. Due to

the different designs and limited number of available studies, it is difficult for studies reporting negative results to assess whether the applied techniques (or study methods) are not sensitive enough to pick up cognitive performance-related alterations, or whether these alterations are not present at all. Interestingly, in those studies where cerebral changes were found, these were most often located in the frontal and/or temporal lobe[17,18,23,28,30,33,34,36,40,61,66-68,71,73-76,81], which is in close agreement with the type of cognitive decline typically experienced in type 2 diabetes.[29] Furthermore, the pathophysiology of cognitive decline in type 2 diabetes is likely to be multifactorial because a number of studies is available that report the involvement of an insulin-mediated mechanism, a glucose-mediated mechanism, vascular effects or inflammatory processes.

Table 2.1 Overview of neuroimaging abnormalities associated with cognitive performance in type 2 diabetes mellitus.

Brain abnormalities	MRI techniques	Major outcomes	References
Clinical applications:			
Atrophy	T1w T2w FLAIR IR	Cerebral atrophy increases with cognitive decline	16-18,20-44
Small vessel disease:			
White matter lesions	T2w FLAIR	White matter lesion load increases with cognitive decline	21,25-27,35,44,45,49,50
Microbleeds	T2*w	No evidence of microbleeds with cognitive decline	25,35,51
Silent brain infarcts	T1w T2w FLAIR	Progression of silent brain infarcts seems related to cognitive decline	45,49,53
Lacunar abnormalities	T1w T2w FLAIR	Cerebral ischemic lesions are related to cognitive decline	25,26,35,40,43,51,55
Impaired cerebral perfusion*	CASL PC-MRI IVIM	Diverse results regarding perfusion in diabetes. Perfusion related to cognitive decline	57-61
Research applications:			
Neuronal dysfunction	fMRI	Reduced fronto-temporal functional connectivity, likely related to cognitive decline	66-76
White matter tract abnormalities	dMRI	Microstructural abnormalities, likely related to cognitive decline	18,30,61,70,78,81-83
Metabolic dysfunction	MRS	Insufficient evidence regarding metabolic alterations and cognitive performance	89,90

Note: only MRI references in combination with cognitive performance were included in this table. T1w, T1-weighted images; T2(*)w, T2(star)-weighted images; FLAIR, fluid attenuated inversion recovery images; IR, inversion recovery images; CASL, continuous arterial spin labeling; PC-MRI, (velocity-sensitive) phase-contrast MRI; IVIM, intravoxel incoherent motion imaging; fMRI, functional MRI; dMRI, diffusion MRI; MRS, magnetic resonance spectroscopy. *Clinically only applied in stroke.

Type 2 diabetes is also known to increase the risk of developing AD.[4,5] MRI studies show that gray matter loss, insulin resistance, and medial temporal lobe atrophy are associated with AD[3,91,92], traits also present in patients with type 2 diabetes.[17,24,25,29,33,43,93] These results suggest that diabetes might be linked to AD and that diabetes and AD might share similar mechanisms underlying cognitive decline. More about the interaction between type 2 diabetes, cognitive decline, and AD is provided in a recently published review.[62]

When alterations in macrostructure are observed, it is safe to assume that these are indicative of macrostructural irreversible endpoints of a pathophysiological cascade. However, changes in perfusion, function, microstructure, and metabolism can be transient phenomena, indicative of on-going potentially pathological processes. The magnitude and direction determine whether these changes should be interpreted as dysfunctional or compensatory. For example, if functional connectivity in a subject with cognitive decrements is reduced, it can be indicative of an underlying dysfunctional process, conversely, for increased connectivity a compensatory mechanism can be plausible.[94]

Future outlook

As the neuronal mechanisms underlying cognitive decline associated with type 2 diabetes still remain to be elucidated, and studies using more-advanced and potentially more-sensitive MRI techniques are scarce, intensified research is needed to investigate the underlying mechanisms of brain damage.[95] It will also be interesting to investigate the role of the neuronal mechanisms underlying cognitive decline in pre-diabetic stages such as the metabolic syndrome or impaired glucose mechanism.[96]

MRI-derived insights on the mechanisms underlying type 2 diabetes related cognitive decline are provided when imaging measures can be associated with blood marker levels (e.g. vascular, insulin, glucose or inflammatory markers). The MRI measure will give additional information on the location and extent of the pathophysiological process and what cerebral region and/or network is sensitive to the effects mediated by the mechanism. A more direct approach to obtain insight into a mechanism is by applying MRI techniques that directly target the cerebral component pertaining to a specific mechanism. For instance, vascular MRI techniques include perfusion based techniques such as ASL, IVIM and phase-contrast MRI. Glucose can directly be measured using ^{13}C-MRS or high-field ^{1}H-MRS. Unfortunately, for insulin and inflammation no cerebral MRI techniques are readily available. However, for those, positron emission tomography (PET) might provide an alternative: neuroinflammation can be measured robustly using the translocator protein microglial marker.[97] And preclinical studies show promising results regarding imaging of insulin receptors by PET using iodine-124 labeled insulin.[98] Additionally, an alternative method to assess

changes in cerebral glucose metabolism is Fluorodeoxyglucose (FDG) PET, which yields estimates of the regional cerebral glucose metabolic rate (CMRglu).[99]

In addition to the imaging techniques discussed in this review, other novel MRI approaches might also yield interesting new biomarkers, such as Dynamic Contrast Enhanced MR Imaging (DCE-MRI), which is an MRI technique where T1-weighted scans are acquired dynamically after injection of a contrast agent, and pharmacokinetic modeling of the enhancing tissue signal can provide information about physiological tissue characteristics, including blood-brain barrier (BBB) integrity in terms of leakage of contrast medium.[100] It could be relevant to study the role of BBB in diabetes, because disruption of the BBB is also considered to be a result of cSVD, and BBB studies on patients with type 2 diabetes are scarce.

Furthermore, metabolites that are relatively difficult to detect, such as GABA, dedicated MRS spectral editing sequences exist to identify and quantify these metabolite concentrations.[86] The use of a specifically designed MRS acquisition scheme allows for the selective recording of signals only from the desired metabolite, while other metabolites are eliminated.

Another important direction is the application of high field MRI[101], as most studies in this review were performed at 1.5T. High field MRI (≥3T) has several benefits as it provides higher spatial resolution and improved SNR ratio, although it is more susceptible for artifacts. Additionally, future studies should incorporate a multiparametric approach, to provide a more complete picture of the locations and nature of affected cerebral regions. Also, analysis approaches for fMRI and dMRI should focus on cerebral networks, as cognitive functions affected by diabetes correspond to networks, rather than localized brain regions.

Clinical relevance

The application of neuroimaging techniques to study diabetes associated accelerated cognitive decline is relevant as we expect to obtain new insights regarding affected brain regions, networks, and tissue abnormalities. Furthermore, MRI measures might provide potential early biomarkers for cognitive decline (see Table 2.1 for an overview), and could potentially be used to identify patients at risk. Please note though that the included studies describe groups and that present MRI techniques are potentially not yet sensitive enough to use as a diagnostic/prognostic tool in individuals. Follow-up studies can be performed to confirm that subjects with sufficient cerebral MRI alterations eventually develop cognitive problems, and one could consider an interventional study with a combination of diet, exercise or medication (such as antihypertensive or antiplatelet drug, improvement of hyperglycemia, intranasal insulin administration)[71] to explore whether cerebral MRI alterations also delay, or even improve, after intervention. Hence, by performing advanced neuroimaging, a more

complete picture can be obtained of the effect of diabetes on the brain, it might provide a better timing of (preventive) therapy, and it could shed some light on the course and efficacy of the therapy to prevent cognitive decline.

Conclusions

Cognitive decline in type 2 diabetes is associated with brain alterations, which can be detected using neuroimaging. Studies investigating the underlying mechanism are limited but point at multifactorial processes (insulin-mediated, glucose-mediated, vascular, and/or inflammatory). The battery of MRI techniques available to study this topic is highly versatile, and several aspects of brain function and integrity can be studied noninvasively. Advanced, novel MRI techniques are expected to reveal more subtle brain alterations compared with only structural MRI. Therefore, more-advanced multiparametric MRI techniques should be implemented in future studies to investigate the role of diabetes on cognitive performance, and the underlying pathophysiological mechanisms.

Literature search

We searched PubMed for articles published until January 4, 2016, with the following terms and combinations of these terms:

"arterial disease", "arterial spin labeling", "atrophy", "axon damage", "brain", "cerebral", "cogniti*", "connectivity", "diabet*", "diffusion tensor imaging", "DTI", "fMRI", "functional MRI", "imaging", "lacun*", "lacunar infarct", "microbleeds", "microstructural abnormalit*", "MRI", "MRS", "MR spectroscopy", "neuronal dysfunction", "neuronal function", "neuropathy", "perfusion", "syndrome", "type 2", "vessel disease", "white matter lesion".

We included articles identified from these searches and relevant references cited in the articles.

The neuropsychological terminology is subdivided in 1) (verbal) memory, 2a) (information) processing speed, 2b) sensory speed, 2c) motor speed, 3) IQ, 4) global cognition, 5) attention functions, 6) executive functions, 7) psychomotor functions, 8) visuoconstruction, and 9) fluency, according to Hebben and Milberg.[102] Speed is subdivided into three components: 1) processing speed (central part/brain), 2) sensory speed (visual aspects) and 3) motor speed (conducting part of a test/trail).

Animal studies, studies on patients with type 1 diabetes mellitus, and studies in which MRI results were presented without addressing correlations with cognitive performance were not included. Only articles written in English were included.

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Chapter 3

Altered hippocampal white matter connectivity in type 2 diabetes mellitus and memory decrements

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Abstract

Type 2 diabetes mellitus is associated with cognitive decrements. Specifically affected cognitive domains are learning and memory, for which the hippocampus plays an essential role. The pathophysiological mechanism remains to be revealed. The present study examined whether local hippocampal microstructure and white matter connectivity are related to type 2 diabetes and memory performance. Forty participants with type 2 diabetes and 38 participants without type 2 diabetes underwent detailed cognitive assessment and 3-Tesla diffusion magnetic resonance imaging (MRI). Diffusion MRI was performed to assess microstructure (fractional anisotropy and mean diffusivity) and white matter connectivity (tract volume) of the hippocampus, which were compared between participants with and without type 2 diabetes. No differences in hippocampal microstructure were observed. Participants with type 2 diabetes had fewer white matter connections between the hippocampus and frontal lobe ($p=0.017$). Participants who scored lower on memory function, regardless of type 2 diabetes, had fewer white matter connections between the hippocampus and temporal lobe ($p=0.017$). Taken together, type 2 diabetes and memory decrements appear to be associated with altered hippocampal white matter connectivity.

Introduction

Type 2 diabetes mellitus is associated with cognitive decrements[1], accelerated cognitive decline[2], and an increased risk for developing dementia and Alzheimer's disease.[2-5] In particular, learning and memory are the most prominently and specifically affected cognitive domains.[6,7] It is well known that the hippocampus plays an essential role in learning and memory processes. Previous studies, which focused on conventional structural magnetic resonance imaging (MRI), demonstrated hippocampal atrophy in type 2 diabetes patients with memory problems.[8-10] Using functional MRI, Zhou et al.[11] showed reduced functional connectivity between the hippocampus and other parts (i.e. frontal, temporal, and parietal) of the brain. Therefore, there is a need to investigate whether the intrinsic hippocampal microstructure and the white matter connectivity to other brain regions are affected in type 2 diabetes and related to memory decrements.

Diffusion MRI (dMRI) is a noninvasive advanced MRI technique that provides greater insights into cerebral white matter abnormalities (i.e. microstructure and white matter connectivity) by measuring the hindered diffusion of water molecules. The most commonly used diffusion measures are: *i*) fractional anisotropy (FA), which describes the preferred diffusion directionality of water molecules and alterations in the microstructural organization of the white matter, and *ii*) mean diffusivity (MD), which represents the mean magnitude of water diffusivity and reflects tissue density.[12,13] In addition, the spatial organization of white matter fiber bundles (i.e. tract volume) between brain regions can be derived from the directional information of the diffusing water molecules. Note that the intrinsic microstructure (FA and MD) and white matter connectivity measures are not completely independent properties. For example, locally increased FA focuses the directionality of white matter tracts and might therefore influence the connectivity. However, because the two concepts describe different aspects of microstructure, it is relevant to report on both.

Previous dMRI studies on type 2 diabetes demonstrated reduced FA and increased MD in different brain regions[14-16] or in white matter tracts connecting frontal, parietal, and temporal regions.[17] Other type 2 diabetes studies have related increased MD in the parahippocampal gyrus to lower memory performance[18] and reduced FA in the cingulum bundle to higher memory performance.[19] To identify microstructural correlates of cognitive decrements, it is important to focus on white matter connectivity, because cognitive function depends on the transfer of information between different brain regions via white matter fiber bundles. Besides the commonly used local diffusion measures of specific white matter fiber bundles, it is important to investigate whether white matter connectivity (i.e. total tract volume of white matter fibers) differs and correlates with cognitive performance in type 2 diabetes.

To the best of our knowledge, there are no type 2 diabetes studies that focus specifically on the hippocampus and memory performance using (hippocampal) white matter connections. Therefore, the present study aimed to examine whether

hippocampal microstructural abnormalities and white matter connections are related to type 2 diabetes and memory performance.

Materials and methods

Study population

Forty-seven participants with type 2 diabetes and 41 participants without type 2 diabetes were recruited from the first 866 participants of The Maastricht Study for additional brain MRI measurements. The Maastricht Study is an ongoing observational prospective population-based cohort study that focuses on the etiology, pathophysiology, complications and comorbidities of type 2 diabetes. Participants are aged between 40-75 years and live in the southern part of the Netherlands.[20] Participants are considered to have diabetes according to the WHO 2006 criteria if they use diabetes medication, or if they have a fasting blood glucose ≥7.0 mmol/l, and/or a 2-h blood glucose ≥11.1 mmol/l after an oral glucose tolerance test. Participants without type 2 diabetes are characterized by fasting blood glucose <6.1 mmol/l and a 2-h blood glucose <7.8 mmol/l. At baseline inclusion, participants underwent an extensive battery of measurements, including cognitive performance tasks, blood pressure measurements, and blood sampling. A detailed overview of all procedures is provided in Schram et al..[20] After the baseline measurements of The Maastricht Study, participants were invited to participate in the present MRI study.

Participants with the highest and lowest cognitive scores were selected from the first 866 participants to increase the probability of finding MRI differences associated with cognitive decrements (Table 3.1). A detailed selection procedure is provided in van Bussel et al..[21] In brief, the division of participants in a low and high cognition group was based on a cumulative score of three neuropsychological tests covering the domains of verbal memory, attention and flexibility, and executive functioning (Table 3.1). Scores were adjusted for age, sex, and education level using linear regression. Exclusion criteria for participants were: (A) a known history of stroke or neurological disease; (B) a time span between enrollment in The Maastricht Study and MRI >1.5 years; (C) incomplete cognitive assessments; (D) type 1 diabetes mellitus; (E) an impaired fasting blood glucose level in participants without type 2 diabetes; (F) mild cognitive impairment or dementia; (G) the metabolic syndrome; (H) color blindness; and (I) unknown diabetes status. The low and high cognition groups were matched on age, sex, and education level, and display a similar distribution of participants with and without type 2 diabetes (Table 3.1).

After taken into account those individuals who declined the invitation and exclusion of participants with MRI contraindications, a total of 47 and 41 participants with and without type 2 diabetes were included, respectively.

Prior to MRI, these participants underwent a general cognitive function test (Mini-Mental State Examination, MMSE[22]) to assess clinically significant differences in cognitive performance compared with the baseline cognitive tests at enrollment in The Maastricht Study. Structural and dMRI brain scans were obtained from all participants. The study was approved by the Medical Ethics Committee of the Maastricht University Medical Center (MUMC+), the Netherlands, and all participants provided their written informed consent. The study is registered at http://www.clinicaltrials.gov (with identifier NCT01705210).

Table 3.1 Characteristics of the two cognition groups.[a]

	Lower cognition (n = 40)	Higher cognition (n = 38)	p-value
Type 2 diabetes (%), n	55.0 (n = 22)	47.4 (n = 18)	0.500[b]
Age (years)	61.1 ± 9.5	62.7 ± 6.7	0.367
Sex, male (%), n	57.5 (n = 23)	52.6 (n = 20)	0.666[b]
Education			0.769[b]
Low (%), n	15.0 (n = 6)	21.1 (n = 8)	
Middle (%), n	47.5 (n = 19)	42.1 (n = 16)	
High (%), n	37.5 (n = 15)	36.8 (n = 14)	
WLT total score	37.1 ± 10.0	50.1 ± 9.0	<0.001
Stroop (sec)	63.3 ± 35.2	34.9 ± 13.1	<0.001
Verbal fluency	20.3 ± 4.9	27.3 ± 5.7	<0.001
Cumulative cognition score	-2.30 ± 2.18	2.10 ± 1.25	<0.001

Data are mean ± SD. WLT, (verbal memory) Word Learning Test. [a]Only participants who were included in the final analysis; Independent samples *t*-test; [b]Pearson χ^2-test.

Magnetic resonance imaging

MRI data were acquired on a 3T scanner (Achieva TX, Philips Healthcare, Best, the Netherlands) using a 32-element head coil for parallel imaging. The MRI protocol consisted of structural scans for neuroradiological evaluation (including T1-, T2-, T2*-weighted and fluid attenuated inversion recovery (FLAIR) sequences) and high angular resolution diffusion imaging (HARDI). A three-dimensional T1-weigthed (T1) fast field echo sequence (TR/TE 8.1/3.7 ms, 1.00 mm isotropic voxel size, 170 continuous slices, matrix size of 240 x 240; 7:56 min acquisition time) and FLAIR (TR/TE/TI 4800/276/1650 ms, 1.12 mm isotropic voxel size, matrix size of 224 x 224; 4:53 min acquisition time) were acquired. HARDI data were obtained using an echo-planar imaging (EPI) sequence (TR/TE 6980/84 ms, 2.4 mm isotropic voxel size, 128 diffusion sensitizing gradient directions, a *b* value of 1500 s/mm^2; 15:56 min acquisition time). In addition, a single minimally diffusion-weighted image (b0-scan) was acquired.[23]

Data analysis

The T1 images were automatically segmented to obtain both hippocampal volumes, intracranial volume, subcortical gray matter, and the cortical areas using FreeSurfer (Martinos Center for Biomedical Imaging, Boston, MA, USA)[24] and the segmentations

were inspected visually. dMRI data analysis (preprocessing, tractography, connectivity analyses) was performed with the diffusion MR toolbox ExploreDTI version 4.8.2.[25] In brief, the preprocessing steps included: *i*) visual image quality assessment; *ii*) correction of dMRI images for eddy current induced geometric distortions and head motion; *iii*) correction of dMRI images for EPI distortions and transformation to T1 space; and, finally, *iv*) estimation of the diffusion tensor for calculating the FA and MD maps.

After preprocessing, the local diffusion measures (FA and MD) were extracted from both hippocampi as derived from FreeSurfer. Subsequently, fiber orientation distributions (FOD) were estimated using constrained spherical deconvolution (CSD) with a maximum harmonic degree of 8[26], which allows fiber tracking through regions with crossing fibers. The FOD represents the local fiber orientation. Whole brain probabilistic tractography was performed using FOD sampling[27] with a seed point resolution of 1 mm^3, a step size of 1 mm, and an FOD and maximum deflection angle threshold of 0.1 and 30˚, respectively, yielding approximately 4.3 million streamlines for each dataset. Next, connectivity analysis was performed to obtain white matter tracts (tract volumes) from the individually segmented hippocampi, used as seed region (i.e. include tracts that only go through the hippocampus), to the segmented gray matter. The segmented gray matter was subdivided into five regions: frontal lobe, parietal lobe, temporal lobe, occipital lobe, and subcortical gray matter.[28,29] Examples of white matter tracts between both hippocampi and the frontal lobe are provided in Figure 3.1. Subsequently, the tract volumes seeded from both hippocampi to each region were normalized to the intracranial volume to reduce inter-individual variation.[30] A previous study from our group showed that white matter tracts (tract volumes) are reproducible.[31] The local diffusion measures (FA and MD) were averaged and tract volumes were added up over the left and right hippocampus, respectively. Tract volumes are interpreted as a measure for connectivity.[32]

After careful analyses, data from forty type 2 diabetes participants and 38 participants without type 2 diabetes remained suitable for final analysis. Data from nine participants were excluded as a result of incomplete data (n=1), claustrophobia (n=2), nondiabetes participants with impaired fasting blood glucose levels (n=2), Parkinsonism (n=1), brain injury because of an accident (n=1), an incidental finding (i.e. tumor, n=1), and susceptibility artifacts (n=2).

Cognition

As described in Schram et al.[20], participants completed an extensive cognitive battery at enrollment in The Maastricht Study. Global cognitive functioning was measured using the MMSE. Verbal memory was assessed by the 15-Word Learning Test (WLT) total score, in which 15 words are presented in five subsequent trials. Immediately after each trail, participants recall as many words as they are able. The maximum score that could be reached, was 75.

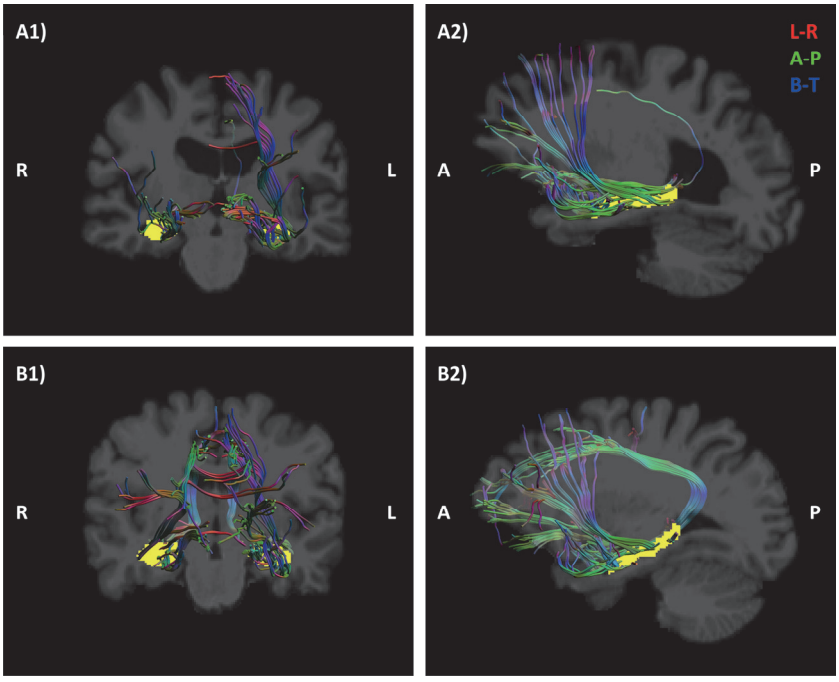

Figure 3.1 White matter fiber bundles seeded from both hippocampi (yellow regions of interest) to the frontal lobe of representative participants with (A) and without (B) type 2 diabetes (**A1** and **B1**, coronal view; **A2** en **B2**, sagittal view), projected on a semitransparent T1-weighted image. The colors of the tracts represent the left-right (red), anterior-posterior (green), and bottom-top (blue) directions. Depicted coronal and sagittal levels are purely for illustrative purposes; quantitative analyses was performed independent of the visual presentation selected.

Covariates

Educational level was assessed by interview and classified into eight levels commonly used in the Netherlands: *1*) no education; *2*) primary education; *3*) lower vocational education; *4*) intermediate general secondary education; *5*) intermediate vocational education; *6*) higher general secondary education; *7*) higher vocational education; and *8*) university degree. For the present study, educational level was subdivided into three groups: low (level 1-3), middle (level 4-6), and high (level 7-8). Office blood pressure was assessed three times on the right arm after a 10-min rest, using a noninvasive blood pressure monitor (Omron 705IT, Omron, Kyoto, Japan). A fourth measurement was performed when the difference between measurement two and three exceeds more than 10 mmHg. Here, we used the averaged blood pressure values over all the available measurements.[20] White matter lesions were automatically segmented using T1 and FLAIR in accordance with the method described by de Boer et al..[33]

Statistical analysis

Descriptive participants' characteristics and diffusion measures are reported as the mean ± SD. Group characteristics were tested using independent samples *t*-tests and Pearson χ^2-tests using SPSS, version 20 (IBM Corp., Armonk, NY, USA).

Linear regression analyses, adjusted for age, sex, education level, body mass index, systolic blood pressure, relative (to intracranial) hippocampal volume, and relative white matter lesion load to correct for differences in clinical characteristics between groups, were performed to assess the association of the hippocampal diffusion measures (FA, MD, and tract volumes) with type 2 diabetes status and memory performance. In addition, a correction for multiple testing was applied according to Benjamini and Hochberg[34] using a false discovery rate of 10%.

To investigate the combined effect of type 2 diabetes and memory on the diffusion measures, an interaction term between type 2 diabetes and memory performance (WLT total score) was added to the same linear regression model. The use of continuous fasting blood glucose and HbA_{1c} values instead of the dichotomous diabetes status was also tested.

Results

Table 3.1 shows the baseline characteristics of the low and high cognition groups because participants were selected based on cognitive status. The groups were matched on age, sex, and education and participants with type 2 diabetes were divided equally over the two groups. Table 3.2 shows the clinical characteristics of participants with type 2 diabetes. Type 2 diabetes participants had higher fasting blood glucose levels, higher HbA_{1c} levels, higher body mass index, higher diastolic and systolic blood pressure, and larger white matter lesion loads, compared with healthy controls (Table 3.2). With respect to cognition, type 2 diabetes participants scored significantly lower on the WLT total score (p=0.004) and on baseline MMSE score (p=0.008) compared with nondiabetes participants. Baseline and repeated MMSE did not differ between participants with and without type 2 diabetes (p=0.317).

For the hippocampus, the local FA was decreased in type 2 diabetes compared with nondiabetes participants (0.11±0.01 and 0.12±0.02, respectively; p=0.033), whereas the MD was increased in type 2 diabetes participants compared with nondiabetes participants (0.98±0.06 and 0.93±0.05 10^{-3} mm^2/s, respectively; p<0.001). However, after adjustment for covariates, multivariable linear regression revealed no significant associations between any of the local diffusion measures and type 2 diabetes or memory performance.

Table 3.2 Clinical characteristics of participants with type 2 diabetes.

	Participants with type 2 diabetes (n = 40)	Participants without type 2 diabetes (n = 38)	p-value
Type 2 diabetes-related variables			
Duration of diabetes (years)	9.9 ± 6.8	-	
Fasting blood glucose (mmol/l)	7.5 ± 1.2	5.1 ± 0.3	<0.001
HbA_{1c} (%)	6.7 ± 0.5	5.6 ± 0.4	<0.001
HbA_{1c} (mmol/mol)	50.3 ± 4.9	38.1 ± 4.4	<0.001
Type 2 diabetes medication			
None (%)	12.5	100	<0.001[a]
Oral medication (%)	75.0	-	
Insulin (%)	2.5	-	
Insulin and oral medication (%)	10.0	-	
Clinical variables			
BMI (kg/m^2)	29.2 ± 3.5	24.7 ± 2.9	<0.001
SBP (mmHg)	152 ± 18	131 ± 18	<0.001
DBP (mmHg)	83 ± 11	76 ± 13	0.014
White matter lesion volume (cm^3)	6.0 ± 10.5	2.0 ± 1.5	0.021
Left hippocampal volume (cm^3)	3.8 ± 0.5	4.0 ± 0.4	0.076
Right hippocampal volume (cm^3)	4.0 ± 0.5	4.1 ± 0.4	0.332
Cognitive score			
Baseline MMSE total score	28.6 ± 1.5	29.4 ± 0.9	0.008
WLT total score	39.8 ± 10.8	47.2 ± 11.1	0.004

Data are mean ± SD. HbA_{1c}, glycated hemoglobin; BMI, body mass index, SBP, systolic blood pressure; DBP, diastolic blood pressure; (baseline / repeated) MMSE, (The Maastricht Study / before MRI) Mini-Mental State Examination; WLT, (verbal memory) Word Learning Test. Independent samples *t*-test; [a]Pearson χ^2-test.

Figure 3.2 shows quantitative boxplots of the relative tract volumes seeded from both hippocampi to the various brain regions. Tract volumes from the hippocampi to the frontal lobe (p=0.005), temporal lobe (p=0.010), and subcortical gray matter (p=0.031) were decreased in type 2 diabetes participants. After adjustment for covariates, multivariable linear regression (Figure 3.2 and Table 3.3) revealed a decreased relative tract volume from the hippocampi to the frontal lobe in type 2 diabetes participants (p=0.017, which remains significant after correction for multiple testing). For participants (regardless of type 2 diabetes) who scored lower on memory performance, multivariable linear regression (Table 3.3) revealed a decreased relative tract volume to the temporal lobe (p=0.017, which remains significant after correction for multiple testing).

Additional multivariable linear regression including the interaction term (type 2 diabetes times WLT total score) showed no significant interaction (p>0.15). Analyses with fasting blood glucose levels and HbA_{1c} both showed a trend: high fasting blood glucose levels and high HbA_{1c} levels are associated with a decreased relative tract volume from the hippocampi to the frontal lobe (p=0.069 and p=0.081, respectively).

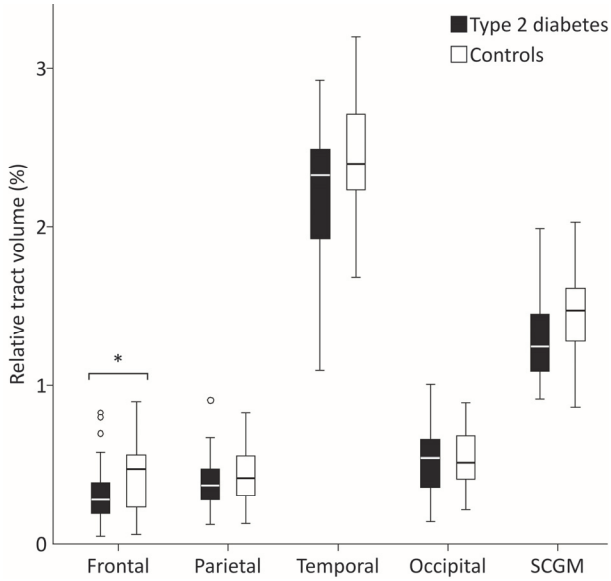

Figure 3.2 Boxplots of the relative hippocampal tract volumes to the frontal lobe, parietal lobe, temporal lobe, occipital lobe, and subcortical gray matter (SCGM) between participants with and without type 2 diabetes. *Tract volumes were significantly different between groups after multivariable linear regression analysis (p<0.05, Table 3.3).

Table 3.3 Relationship between relative (to intracranial) white matter connections (tract volumes) seeded from both hippocampi and type 2 diabetes status and memory performance.

	Hippocampus					
relative	Type 2 diabetes status			Memory performance		
tract volumes	β	95% CI	p-value	β	95% CI	p-value
To frontal lobe	-0.720	-1.310 to -0.130	0.017*	-0.105	-0.356 to 0.146	0.408
To parietal lobe	-0.517	-1.124 to 0.089	0.093	-0.268	-0.527 to -0.010	0.042
To temporal lobe	0.118	-0.332 to 0.567	0.603	0.235	0.043 to 0.427	0.017*
To occipital lobe	0.022	-0.549 to 0.594	0.938	-0.137	-0.380 to 0.107	0.267
To SCGM	-0.024	-0.487 to 0.438	0.916	-0.081	-0.278 to 0.116	0.416

Standardized β (95% confidence interval (CI)) indicates increments/decrements of the tract volumes with type 2 diabetes status or memory performance. SCGM, subcortical gray matter. Model: adjusted for age, sex, education level, body mass index, systolic blood pressure, corresponding relative (to intracranial) hippocampal volume, and relative (to intracranial) white matter lesion volume. *Significant after correction for multiple comparisons.

Discussion

The present study investigated whether hippocampal microstructural abnormalities are related to type 2 diabetes and verbal memory. To our knowledge, this is the first study to investigate white matter connections from the hippocampus to different brain lobes in type 2 diabetes. The results of the present study demonstrate fewer white matter connections to the frontal lobe in participants with type 2 diabetes compared with nondiabetic participants. For participants who scored relatively low on memory performance, we observed fewer white matter connections to the temporal lobe.

In the present study, participants with type 2 diabetes revealed decreased white matter connections between the hippocampi and the frontal lobe. As a result of decreased white matter connectivity, the white matter appears to be less well organized to transfer and integrate information in participants with type 2 diabetes. Therefore, the affected white matter connectivity, rather than the intrinsic microstructure of the tracts in participants with type 2 diabetes, might underlie the memory decrements. The fact that the frontotemporal connection was affected is in close agreement with the type of cognitive decrements (i.e. memory, executive functioning, processing speed) in type 2 diabetes[8], as well as previous studies reporting frontal and/or temporal structural alterations.[11,35]

Only two other studies[17,19] have previously investigated white matter connectivity (tract volumes) in participants with type 2 diabetes and focused, not on the hippocampal, but other white matter tracts (including the superior longitudinal fasciculus and the uncinate fasciculus), for which they did not observe any differences in volume. Hoogenboom et al.[19] related cognitive decline to reduced FA of the uncinate fasciculus bundle. Reijmer et al.[17] showed microstructural abnormalities in specific white matter bundles. Another study by Reijmer et al.[36] observed disruptions of the more global white matter network in patients with type 2 diabetes, which was related to cognitive decline. The decreases in hippocampal white matter connections in type 2 diabetes observed in the present study likely contribute to those global network disruptions.

For participants who scored relatively low on memory performance, we observed fewer hippocampal white matter connections to the temporal lobe. The temporal lobe plays an important role in memory and our results potentially indicate that there is less optimal transfer and integration of information between the hippocampus and the temporal lobe, which could play a role in the underlying memory decrements. The involvement of the temporal lobe in memory performance that we observed is in line with studies by Yau et al.[18,37], who showed that white matter abnormalities in the temporal stem (decreased FA) and parahippocampal gyrus (increased MD) explained the lower memory performance in type 2 diabetes. It was concluded that type 2 diabetes has a deleterious effect on the vulnerability of the temporal lobe memory networks[37], especially the hippocampus and parahippocampal gyrus.[18]

The intrinsic microstructure measures (FA and MD) were not different between participants with and without type 2 diabetes or were not associated with memory performance after controlling for the study characteristics. Falvey et al.[15] observed increased MD for type 2 diabetes in both hippocampi after controlling for age, sex, and race. When using the same statistical model as Falvey et al.[15], we observed similar results (i.e. increased hippocampal MD in participants with type 2 diabetes; p=0.014), which illustrates the importance of the inclusion of clinical characteristics as covariates to prevent superfluous results. Previously, in the same population, we showed an association between increased hippocampal MD and memory performance, but not specifically for type 2 diabetes, for which we found differences in microvasculature.[21] In that study, we applied intravoxel incoherent motion imaging, another diffusion technique, which distinguishes water diffusion of the intrinsic microstructure (parenchyma) from that of the microvasculature. Such a distinction cannot be made using standard dMRI. Other studies have also reported local microstructural white matter abnormalities in adolescents with type 2 diabetes[16] and in patients with type 2 diabetes[14]; however, these results were not specific to the hippocampus. Other nondiabetic studies, specific to the hippocampus, have shown associations between higher hippocampal MD and memory performance in nondemented participants and in elderly participants with cerebral small vessel disease, respectively.[38,39]

In the present study, type 2 diabetes participants scored lower on memory performance and had less hippocampal white matter connectivity to the frontal lobe. However, we did not observe a synergistic effect (interaction) of type 2 diabetes and memory decrements on the white matter connectivity between the hippocampus and frontal lobe. This might be attributable to the relatively healthy diabetes population engaged in the present study, which showed no obvious abnormalities or volume reductions of the hippocampus (Table 3.2) and was only mildly affected in terms of memory performance, and may be under good treatment control regarding glucose levels. Moreover, the memory scores of participants with type 2 diabetes were in the range of normal performance[40] and hence potentially not sufficiently strong to detect a synergistic effect of type 2 diabetes and memory decrements on the white matter connectivity between the hippocampus and the frontal lobe. In addition, it might be that other connections, either involving the hippocampus or not, are related to memory performance, although these connections were not considered in the present study. The observed effect might therefore represent relatively early signs of developing brain abnormalities related to type 2 diabetes and decrements in cognition.

The strengths of the present study include: first, the extensive characterization of the participants. Second, both hippocampi that were used as seed for the connectivity analyses and the cortical areas were automatically (and thus operator-independent) parcellated with FreeSurfer, which reduces the risk and variability of anatomical misplacements. Third, the scan protocol included high quality structural and dMRI data. The dMRI data were obtained using a high number (128) different gradient directions.

Fourth, whole brain tractography yielded approximately 4.3 million tracts, which were used for the connectivity analyses from the hippocampus to the rest of the brain.

A number of issues limit the conclusions. First, the study had a cross-sectional design. Therefore, the results should be interpreted cautiously. Nevertheless, the initial results are promising and open directions for future (longitudinal) studies. Second, the time span between enrollment for The Maastricht Study (i.e. baseline, in which cognitive tests were performed) and the subsequent MRI assessment was 16.4±3.1 months, which might have limited the validity of the subject characteristics and the long-term validity of the WLT score at the time of the MRI evaluation. Nevertheless, the baseline and repeated MMSE did not differ, which is indicative of no clinically significant cognitive differences within this time frame. Third, tract volume was used as measure for white matter connection.[32] In theory, alterations in axon diameters or myelination can also lead to differences in tract volume at the same time as maintaining the number of actual connections, although this explanation is less likely. Finally, the present study does not consider specific tracts, such as the cingulum bundle or the uncinate fasciculus[17,19], and therefore cannot provide details of specific effects on these tracts. However, the applied approach, which considers all connections from the hippocampus to other cerebral regions, facilitates a more global assessment of hippocampal connectivity, which might be more sensitive to effects of type 2 diabetes and cognition.

Future longitudinal studies will provide additional insights, favorably including not only participants with type 2 diabetes, but also potentially participants with pre-diabetes, such as the metabolic syndrome or diabetes with mild cognitive impairment. Furthermore, better memory performance in type 2 diabetes has been shown by improvement in fasting plasma glucose levels.[41] An important question to be addressed in future (longitudinal) studies is whether improvement in glycemic control leads to less affected white matter connectivity, and whether specific tracts (e.g. cingulum) are affected. These extensions could clarify whether the decrease in white matter connectivity is possibly an early brain tissue biomarker for verbal memory decrements.

In conclusion, dMRI tractography revealed reduced white matter connectivity between the hippocampus and the frontal lobe in type 2 diabetes. For participants who scored lower on memory performance, tractography revealed reduced white matter connectivity between the hippocampus and the temporal lobe. Memory decrements in participants with type 2 diabetes appear to be associated with altered hippocampal white matter connectivity. The findings of the present study contribute to a better understanding of diabetes-associated memory performance, although the exact mechanism remains to be revealed in future studies.

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Chapter 4

On the interplay of microvasculature, parenchyma, and memory in type 2 diabetes

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Abstract

Objective

Type 2 diabetes is associated with accelerated cognitive decline, especially regarding memory for which the hippocampus plays an essential role. The pathophysiological mechanisms still remain to be elucidated. The purpose of this study is to examine whether hippocampal microvascular and microstructural changes are related to type 2 diabetes (based on status or based on fasting blood glucose [FBG] levels) and verbal memory performance.

Research design and methods

Thirty-nine participants with type 2 diabetes (64.5±6.1 years old) and 34 participants without type 2 diabetes (58.3±9.2 years old) underwent detailed cognitive assessments and 3-Tesla MRI using intravoxel incoherent motion (IVIM) MRI. Multivariate regression analyses controlling for age, sex, education level, BMI, systolic blood pressure, hematocrit level, and relative hippocampal volume were performed to examine associations between hippocampal IVIM measures, type 2 diabetes (status and FBG), and memory performance.

Results

For the microvasculature, blood perfusion volume (*f*) was larger in participants with type 2 diabetes, *f* and blood flow (*fD**) increased with higher FBG levels, and microvascular pseudodiffusion (*D**) and *fD**, which are indicative of altered microvasculature, were higher in participants with both relatively high FBG levels and low memory performance. In addition, *fD** increased with lower memory performance. For the parenchymal microstructure, the diffusion (*D*), indicative of injured microstructure, was higher with reduced memory performance.

Conclusions

In addition to the parenchymal microstructure, especially the microvascular properties of the hippocampus are altered in participants with both type 2 diabetes and memory problems and possibly hint at an underlying vascular mechanism.

Introduction

Type 2 diabetes is associated with cognitive deficits, accelerated cognitive decline, and an increased risk of dementia and Alzheimer's disease (AD).[1-3] A prominently affected cognitive domain in type 2 diabetes is memory[4], for which the hippocampus plays an essential role. In patients with type 2 diabetes and memory problems, reduced hippocampal volumes have previously been reported.[5] Furthermore, chronic hyperglycemia has been shown to be associated with altered hippocampal microstructure and memory performance in elderly individuals without diabetes.[6] Unfortunately, the exact mechanism underlying hyperglycemia and memory impairment is not fully understood. In patients with stroke, it was shown that chronic hyperglycemia might be involved in the remodeling of the cerebral microvasculature.[7] Possibly the microvasculature is also affected in hippocampal areas in patients with type 2 diabetes. However, advanced and detailed MRI analyses with respect to hippocampal microstructure (parenchyma) as well as microvasculature to explain memory problems in patients with type 2 diabetes have not been conducted so far.

The noninvasive intravoxel incoherent motion (IVIM) MRI technique, which was introduced by Le Bihan et al.[8], enables a detailed assessment of hippocampal microstructure and microvasculature through *1*) the diffusion coefficient (*D*), which is a measure for neural tissue integrity and reflects the diffusion of water molecules in tissue; *2*) the pseudodiffusion coefficient (*D**), which reflects the incoherent motion of water molecules in the microvasculature; *3*) the perfusion fraction (*f*), a measure for blood perfusion volume; and *4*) blood flow (*fD**). The latter two (*f* and *fD**) are potentially sensitive to microvascular pathology. It has recently been shown by Federau et al.[9] that IVIM in the brain yields clinically relevant parameters, e.g., for acute stroke and gliomas. An interesting advantage of the IVIM technique is that it allows the simultaneous (noninvasive) assessment of tissue microstructure and microvasculature, and therefore the interplay between brain tissue and vessels.

The purpose of this study is to examine whether hippocampal microvascular and microstructural changes, derived from an IVIM MRI measurement, are correlated with type 2 diabetes (based on status or based on fasting blood glucose [FBG] levels), verbal memory decline, and the potential interaction between type 2 diabetes and memory performance.

Research design and methods

Study population

A total of 47 participants with type 2 diabetes and 41 participants without type 2 diabetes were recruited from the first 866 participants (total subject pool) of The

Maastricht Study. The Maastricht Study is an ongoing observational, prospective, population-based cohort study that focuses on the etiology, pathophysiology, complications, and comorbidities of type 2 diabetes. Participants are between 40 and 75 years of age and live in the southern part of the Netherlands.[10] Participants are considered to have diabetes according to the World Health Organization 2006 criteria if they use diabetes medication or if they have an FBG ≥7.0 mmol/l or a 2-h blood glucose ≥11.1 mmol/l. Participants without type 2 diabetes are characterized by FBG <6.1 mmol/l and a 2-h blood glucose <7.8 mmol/l. At baseline inclusion, participants underwent an extensive battery of measurements, including cognitive performance tasks, blood pressure measurements, and blood sampling, for example. A detailed overview is provided in Schram et al..[10] After their baseline measurements of The Maastricht Study, participants were invited to participate in this MRI study.

Participants with a broad range of cognitive performances were selected from the total subject pool to increase the probability of finding MRI differences associated with cognitive decrements (see the Supplementary Data for the detailed selection procedures). This selection was based on a cumulative score of neuropsychological tests: *1*) verbal memory (verbal word learning)[11]; *2*) executive functioning, attention, and flexibility (Stroop test)[12]; and *3*) fluency test.[13] Scores were adjusted for age, sex, and education level using linear regression. Exclusion criteria for participants were: *1*) a known history of stroke or neurological disease, *2*) if the time span between enrollment in The Maastricht Study and MRI assessment was >1.5 years, *3*) incomplete cognitive assessments, *4*) type 1 diabetes, *5*) an impaired FBG level in participants without type 2 diabetes, *6*) mild cognitive impairment (MCI), *7*) participants with the metabolic syndrome, *8*) participants with color blindness, and *9*) participants with an unknown diabetes status. After declining the invitation or exclusion of participants with MRI contraindications, a total of 47 and 41 participants with and without type 2 diabetes were included, respectively.

Prior to MRI, all participants underwent a general cognitive function test (Mini-Mental State Examination [MMSE])[14] to assess clinically significant differences in cognitive performance compared with the baseline cognitive tests at enrollment in The Maastricht Study. Structural and IVIM brain scans were obtained from all participants. This study was approved by the Medical Ethics Committee of the Maastricht University Medical Center (MUMC+), and all participants gave written informed consent. This study is registered at http://www.clinicaltrials.gov with identifier NCT01705210.

Magnetic resonance imaging

MRI data were acquired on a 3T scanner (Achieva TX; Philips Healthcare, Best, the Netherlands) using a 32-element head coil for parallel imaging. The MRI protocol consisted of structural scans for neuroradiological evaluation (including T1-, T2-, and T2*-weighted and fluid attenuated inversion recovery [FLAIR] sequences) and an IVIM scan. A three-dimensional T1-weighted (T1) fast field echo sequence (TR/TE of

8.1/3.7 ms, 1.00 mm isotropic voxel, 170 continuous slices, matrix size of 240 x 240, and 7:56 min acquisition time) was acquired, and IVIM data were obtained using a single-shot spin-echo echo-planar imaging sequence (TR/TE of 6800/84 ms, 2.4 mm isotropic voxel, inversion time of 2230 ms, and 5:13 min acquisition time). Inversion prepulses were applied to suppress the signal contamination of cerebrospinal fluid.[15] Images were acquired at multiple *b* values (0, 5, 7, 10, 15, 20, 30, 40, 50, 60, 100, 200, 400, 700, 1000, and 1500 s/mm^2) in the phase-encoding direction, and the number of signal averages was 1, except for the largest three *b* values, which were 2, 3, and 3, respectively, to improve the signal-to-noise ratio.

Data analysis

The T1 images were automatically segmented to obtain both hippocampal volumes using FreeSurfer.[16] As we expect that microvasculature measures are most strongly indicative of gray matter, analyses were restricted to the gray matter. The IVIM data were preprocessed with the diffusion MR toolbox ExploreDTI (v.4.8.2).[17] In brief, the preprocessing steps included *1*) visual image quality assessment, *2*) eddy current induced geometric distortions and head motion corrections, and, finally, *3*) echo-planar imaging distortion correction.

The IVIM model describes the quantitative relationship between signal intensity and the applied diffusion sensitization (see Supplementary Eq. 1). In this model, the diffusion motion of parenchymal and intravascular water molecules can be distinguished. A quantitative description of the model then provides the following physiological measures (see Supplementary Data): the blood perfusion *f*, the diffusion coefficient of parenchymal water *D*, and the so-called pseudodiffusion coefficient of intravascular water *D**, which resembles both diffusion and convection of blood water.[8] We also consider *fD** (i.e. *f* times *D**), which has previously been shown to be related to the classical cerebral blood flow (CBF).[9,18]

IVIM measures were derived after fitting the IVIM model to a biexponential formula, using a two-step approach as described by Federau et al.[19] (see Supplementary Data for the detailed procedures). A fitting example of the two-step approach is given in Supplementary Figure S4.2.

After careful analyses, data from 39 and 36 participants with type 2 diabetes (for right and left hippocampus, respectively) and 34 participants without type 2 diabetes (both hippocampi) remained suitable for final analysis (see Supplementary Data).

Statistical analysis

Descriptive participant characteristics and IVIM measures are reported as mean ± SD. Group characteristics were tested using independent samples Student *t*-test or Pearson χ^2-tests using SPSS (version 20; IBM Corp., Armonk, NY, USA). Linear regression analyses, adjusted for age, sex, education level, BMI, systolic blood pressure,

hematocrit level, and relative (to intracranial) hippocampal volume, were performed to assess the association of the hippocampal IVIM measures (*f*, *D*, *D**, and *fD**) with type 2 diabetes (model 1, dichotomous status; model 2, FBG levels) and memory performance (total score of 15-word learning [verbal] memory task [WLT total score]). For these analyses, IVIM measures, and anthropometrical and cardiovascular characteristics, which were expressed in different scale units, were standardized to comparable units by *z*-score transformation: z = (subject value – population mean) / population SD.

In addition, the interaction term between type 2 diabetes (status or FBG levels) and cognitive performance (WLT total score) was added to both models, to investigate the possible interaction of type 2 diabetes and memory performance. The resulting interaction term (model 2) was devised to be high in participants with both high FBG levels and poor memory performance.

Additionally, linear regression analyses with glycated hemoglobin (HbA_{1c}) levels instead of FBG in model 2 were performed.

Results

Table 4.1 shows the baseline characteristics of the participants. The groups were different with regard to age, sex, and education. Participants with type 2 diabetes had higher FBG levels; higher HbA_{1c} levels, a measure for long-term blood glucose control; higher BMI, and higher diastolic as well as systolic blood pressure. With respect to cognition, participants with type 2 diabetes scored worse on the WLT total score (p=0.016) and on baseline MMSE (p=0.023), but not on the repeated MMSE (p=0.366), and the recall words learning (verbal) memory task (WLT recall score, p=0.178) compared with participants without type 2 diabetes. Baseline and repeated MMSE did not differ between participants with and without type 2 diabetes (p=0.314), which indicates no signs of severe cognitive decline over a period of 16.7±2.7 months.

Table 4.1 also shows the descriptive hippocampal IVIM measures of the participants. The order of magnitude of the IVIM measures in the current study was consistent with previously published data.[19] Figure 4.1 shows the rather heterogeneous pixel-by-pixel distribution of the IVIM measures in the delineated hippocampus.

Linear regression (Table 4.2, model 1) revealed an increased perfusion fraction *f* for participants with type 2 diabetes status (p=0.022). The perfusion fraction *f* was also increased in participants with lower hematocrit levels (β=-0.252, p=0.047). The diffusion coefficient *D* increased with lower memory performance (p=0.021) (Table 4.2) and age (β=0.344, p=0.018). No significant associations were observed for *D**. Blood flow-related *fD** increased with lower memory performance (p=0.038) (Table 4.2).

Additional linear regression analyses with the interaction term (type 2 diabetes status times memory performance) did not show any significant interaction (data not shown).

Table 4.1 Characteristics of the study population.

	Participants with type 2 diabetes (n = 39)	Participants without type 2 diabetes (n = 34)
Demographic factors		
Age (years)	64.5 ± 6.1	58.3 ± 9.2[b]
Sex, male (%)	74.4	35.3[d]
Education[c]		
Low (%)	20.5	17.6
Middle (%)	56.4	29.4
High (%)	23.1	52.9
Type 2 diabetes-related variables		
Duration of diabetes (years)	9.9 ± 6.7	-
FBG (mmol/l)	7.5 ± 1.2	5.0 ± 0.3[b]
HbA_{1c} (%)	6.8 ± 0.5	5.6 ± 0.4[b]
HbA_{1c} (mmol/mol)	50.4 ± 4.9	38.0 ± 4.6[b]
Type 2 diabetes medication		
None (%)	12.8	100
Insulin (%)	2.6	-
Oral medication (%)	74.4	-
Insulin and oral medication (%)	10.3	-
Clinical variables		
BMI (kg/m^2)	29.1 ± 3.5	24.7 ± 2.7[b]
SBP (mmHg)	152 ± 18	129 ± 17[b]
DBP (mmHg)	83 ± 10	75 ± 13[a]
Hematocrit levels (%)	41.8 ± 3.4	41.7 ± 3.0
Left hippocampal volume (cm^3)	3.8 ± 0.5	4.0 ± 0.4
Right hippocampal volume (cm^3)	4.0 ± 0.5	4.0 ± 0.4
Cognitive score		
Baseline MMSE total score	28.7 ± 1.4	29.4 ± 0.9[a]
Repeated MMSE total score	28.7 ± 1.2	29.0 ± 0.9
WLT total score	40.1 ± 10.7	46.4 ± 11.2[a]
WLT recall score	8.5 ± 2.9	9.5 ± 3.7
Hippocampal IVIM measures		
f (%)	2.89 ± 0.76	2.82 ± 0.80
D (10^{-3} mm^2/s)	0.80 ± 0.03	0.79 ± 0.03
D^* (10^{-3} mm^2/s)	7.53 ± 3.63	8.15 ± 3.47
fD^* (10^{-3} mm^2/s)	0.20 ± 0.11	0.22 ± 0.09

Data are mean ± SD. Baseline/repeated MMSE, The Maastricht Study/before MRI MMSE; DBP, diastolic blood pressure; SBP, systolic blood pressure. [a]Independent samples Student *t*-test, $p<0.05$. [b]Independent samples Student *t*-test, $p\leq0.001$. [c]Pearson χ^2-test, $p\leq0.05$. [d]Pearson χ^2-test, $p\leq0.001$.

Linear regression (model 2) with FBG levels revealed an increased perfusion fraction f with higher FBG levels (p=0.031) (Table 4.2) and in participants with lower hematocrit levels (β=-0.266, p=0.037). The diffusion coefficient D increased with lower memory performance (p=0.025) (Table 4.2) and age (β=0.366, p=0.013). No significant associations were observed for D^*. Blood flow-related fD^* increased with higher FBG levels (p=0.020) and with lower memory performance (p=0.041) (Table 4.2).

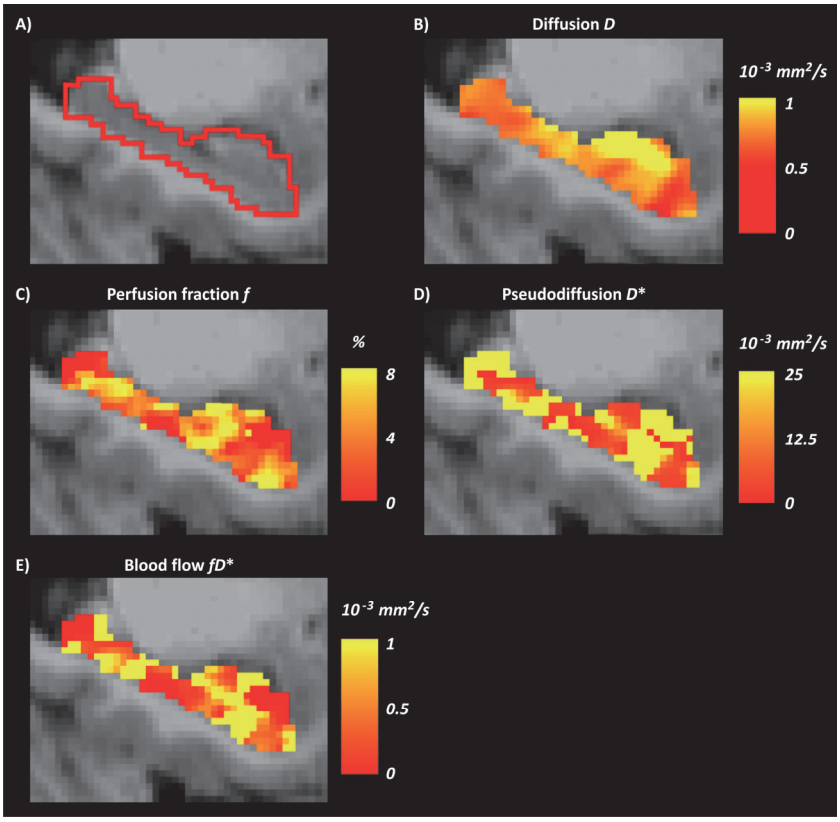

Figure 4.1 (**A**) Sagittal T1 view of the left hippocampus illustrating automated FreeSurfer hippocampal segmentation outlined in red. Pixel-by-pixel analysis of the diffusion coefficient *D* of the gray matter (**B**), the perfusion fraction *f* (**C**), the pseudodiffusion *D** (**D**), and the blood flow-related *fD** (**E**) in a low cognitive-performing participant with type 2 diabetes.

The interaction analysis (FBG levels times memory performance) revealed a significant interaction for *D** (β=0.273, p=0.038) and *fD** (β=0.278, p=0.018).

Additional analyses with HbA_{1c} did not show any significant associations with IVIM measures.

Table 4.2 Relationship between hippocampal IVIM measures and memory performance in type 2 diabetes (either dichotomous status or FBG level).

	Model 1						Model 2					
IVIM	Type 2 diabetes status			Memory performance			FBG			Memory performance		
measures	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value
f	0.736	0.111 to 1.362	0.022	-0.080	-0.342 to 0.183	0.545	0.315	0.030 to 0.600	0.031	-0.061	-0.325 to 0.202	0.642
D	0.415	-0.201 to 1.031	0.183	-0.306	-0.565 to -0.048	0.021	-0.063	-0.346 to 0.220	0.657	-0.300	-0.562 to -0.039	0.025
*D**	-0.053	-0.737 to 0.630	0.876	-0.246	-0.533 to 0.041	0.091	0.104	-0.205 to 0.413	0.505	-0.245	-0.531 to 0.040	0.091
*fD**	0.405	-0.228 to 1.038	0.206	-0.281	-0.547 to -0.015	0.038	0.333	0.054 to 0.611	0.020	-0.268	-0.525 to -0.011	0.041

β (95% CI) indicates increments/decrements of the IVIM measures with type 2 diabetes status or memory performance (model 1) and with FBG or memory performance (model 2). n=71. The model was adjusted for age, sex, education level, BMI, systolic blood pressure, hematocrit level, and relative (to intracranial) hippocampal volume.

Conclusions

The current study simultaneously examined the microvasculature and parenchymal microstructure of the hippocampus in relation to diabetes, FBG levels, and memory performance in participants with and without type 2 diabetes using IVIM. To the best of our knowledge, this is the first study to investigate the microvascular properties of the hippocampus in participants with type 2 diabetes. For the microvasculature, under the model considered, the blood perfusion volume (*f*) was higher in participants with type 2 diabetes, the blood perfusion volume (*f*) and blood flow (*fD**) increased with higher FBG levels, blood flow (*fD**) increased with poorer verbal memory performance, and pseudodiffusion coefficient of vascular water (*D**) and blood flow (*fD**) were higher in participants with both higher FBG levels and lower memory performance. Regarding the hippocampal parenchymal microstructure, under the model considered, the microstructural diffusion coefficient (*D*) increased with poorer verbal memory performance.

Microvasculature

We demonstrated an increased blood perfusion volume in participants with type 2 diabetes, in participants with higher FBG levels, and an increased blood flow with higher FBG in the hippocampus (Table 4.2). Currently, no other hippocampal perfusion studies are available in participants with type 2 diabetes, and global (whole brain) perfusion studies show inconclusive results.[20,21] Moreover, insulin administration in participants with type 2 diabetes leads to reduced cerebral perfusion in the insular cortex and improved cognitive performance.[22]

A previous study found that patients with MCI had higher hippocampal CBF (which is related to blood flow *fD**)[9] compared with healthy control subjects[23,24], whereas the hippocampal CBF was lower in AD patients with more severe cognitive impairment than MCI patients.[23] Hauser et al.[23] hypothesized that the increased hippocampal CBF in MCI patients may be a result of an early compensatory mechanism, possibly due to inflammation, elevation of neuronal activation, or production of vasodilators.[24] This hypothesis can also be extended to our study results, because the cognitive performance of participants with type 2 diabetes is still better but more similar to the cognitive performance of MCI patients than that of AD patients.

The current study revealed that high FBG levels are associated with increased blood perfusion volume and high blood flow in the hippocampus, suggesting that a glucose mechanism might underlie microvascular alterations. In a recently published study by Crane et al.[25], the authors showed that higher blood glucose levels are associated with a higher risk of developing dementia. As participants with type 2 diabetes are associated with an increased risk of dementia, our results might suggest that the blood perfusion volume might be an early MRI biomarker for dementia and is mediated by vascular pathology. As no participants with dementia or MCI were

included in this study, future studies are needed to fully elucidate the interplay of vascular pathology and blood glucose levels in dementia.

The significantly higher blood perfusion volume in participants with lower hematocrit levels is in accordance with findings by Thomas et al..[26]

Interplay of blood glucose, microvasculature, and memory performance

We observed a higher blood flow with lower memory performance (Table 4.2), and a higher microvascular pseudodiffusion and blood flow in participants with both higher FBG levels and lower memory performance. A higher microvascular pseudodiffusion and blood flow could indicate that the hippocampal microvasculature is altered or more leaky, which is in agreement with previously reported increased blood-brain barrier permeability in patients with type 2 diabetes.[27] To investigate the full extent of leaky microvasculature, future studies using, for example, dynamic contrast-enhanced MRI are warranted.[28]

Microstructure

The increased microstructural diffusion with lower memory performance in the hippocampus, as found in our study, hints at a mechanism where an injured microstructure might underlie the memory decline. These results are consistent with a study by Falvey et al.[29], who observed greater hippocampal mean diffusivity in participants with diabetes.

Kerti et al.[6] showed a significant correlation between lower FBG levels and decreased microstructural diffusion within the hippocampus. Nevertheless, in the current study, no such relationship was observed (Table 4.2), possibly due to the fact that we applied the IVIM technique, distinguishing diffusion of the parenchymal microstructure from that of the microvasculature, whereas Kerti et al.[6] applied diffusion tensor imaging, in which this distinction cannot be made. The observation of an association between microstructural diffusion and age in the hippocampus is in line with results demonstrated by Pereira et al.[30] and suggests that microstructural diffusion is sensitive to age-related degeneration.

Limitations

Several limitations to the study need to be discussed. First, the study has a cross-sectional design. Therefore, it was not possible to investigate whether the higher blood perfusion volume is indeed an early compensatory mechanism and/or whether it will decrease at a later stage. Still, the first results are promising and open directions for future studies. Second, the study population was relatively heterogeneous due to the selection procedure but reflects typical participants with and without type 2 diabetes, and therefore the analyses were corrected for age, sex, and education. After correcting

for covariates, group differences became significant. Third, the time span between enrollment for The Maastricht Study (baseline, in which cognitive tests were performed) and the subsequent MRI assessment was 16.7±2.7 months, which might limit the validity of the subject characteristics and the long-term validity of the WLT score at the time of the MRI evaluation. Nevertheless, the baseline and repeated MMSE did not differ, which is indicative of no clinically significant cognitive differences in this time window. Fourth, to limit the scan time, the diffusion was only measured in one direction (phase-encoding direction). Therefore, we investigated the diffusion properties of the hippocampus only in the gray matter, where diffusion is not or less dependent on the directions compared with the white matter.

Clinical perspectives

Our results show that increased blood perfusion volume (*f*) is associated with high blood glucose and is therefore possibly an early biomarker for hyperglycemia-associated impairment of cognitive performance.[25] Moreover, microvascular pseudodiffusion (*D**) and blood flow (*fD**) are indicative of the added effect of hyperglycemia on memory impairment. Previously, it has already been shown that improvement in glycemic control is associated with improved cognition in participants with type 2 diabetes.[31] Thus, it is plausible that higher FBG levels lead to cerebral microvascular alterations, which could explain the impaired cognitive performance. An important next question to be addressed in future studies is how the IVIM measures relate to cognitive performance in individuals with the metabolic syndrome or impaired glucose metabolism (prediabetic stages).[32] In addition, the results of this study indicate that, next to improvement of hyperglycemia, treatment to improve vascular function (e.g., with antihypertensive or antiplatelet drugs) could be beneficial in patients with type 2 diabetes and impaired cognition. This should be explored in other studies. Future (longitudinal) studies are therefore needed to elucidate whether IVIM measures provide an early biomarker for memory impairment and dementia in the context of (pre)diabetes. Validating these potential biomarkers as indicators of biological alterations might open new avenues to monitor therapeutic/lifestyle interventions for improvement of cognition and prevention of cognitive decline.

Conclusion

This study indicated that especially microvascular properties of the hippocampus are altered in participants with type 2 diabetes and memory problems, which hints at a possible contribution of an underlying vascular mechanism. The IVIM measures have the potential to be good candidates as MRI biomarkers for memory impairment in type 2 diabetes.

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Supplementary data

Selection procedure for MRI

The selection procedure from 866 participants of The Maastricht Study was as follows (Supplementary Figure S4.1):

(A) First, twelve participants were excluded due to incomplete cognitive data.

(B) Subsequently, a cumulative cognition score was calculated based on three neuropsychological tests: *1*) verbal memory (verbal word learning), 2) executive functioning, attention, and flexibility (Stroop test), and 3) verbal fluency test (i.e. the ability to produce as many words as possible in 60 seconds). Per test, the scores were adjusted for age, sex, and education level by linear regression and the cumulative cognition score was calculated by adding the *z*-scores (standardized residuals) of the three neuropsychological tests. To increase the possibility of finding suitable MRI biomarkers, initially we selected two extreme groups using the cumulative cognition score with likely the most significant group differences: the 30% worst and the 30% best cognitively performing individuals, yielding 256 participants per group.

(C) Then, additional exclusion criteria were applied: *1*) participants with a time span of >1.5 years between enrollment in The Maastricht Study and MRI; *2*) participants with type 1 diabetes; *3*) participants with the metabolic syndrome; *4*) an impaired FBG level in participants without type 2 diabetes; *5*) participants with a reported stroke or known neurological disease, such as Alzheimer's or Parkinson's disease; *6*) participants with color blindness; and *7*) participants with an unknown diabetes status, yielding 166 potential participants that could be invited for MRI of whom 95 participants belong to the 30% lowest cumulative cognition score and 71 participants belong to the 30% highest cumulative cognition score.

(D) We invited 136 participants of whom 63 participants declined the invitation due to MRI contraindications (i.e. pacemakers, mechanical heart valve, magnetic dentures plates, or a neurostimulator) (n=5), claustrophobia (n=14), or other reasons (n=44). None of the participants had a MMSE score of ≤24, thus no participants suffered from mild cognitive impairment or worse cognitive condition.

(E) According to this selection thus far, 39 participants with a low and 34 participants with a high cumulative cognition score were enrolled in the study.

(F) To further increase the total number of participants and to achieve a continuous scale in cognition scores, we invited 23 additional participants with an average cumulative cognition score. Seven out of these 23 participants declined the invitation due to MRI contraindications (i.e. pacemaker) (n=1), changed diabetes status (n=1), or other reasons (n=5). None of the 15 additional participants had a MMSE score of ≤24. These 15 participants (11 participants with type 2 diabetes and 4 without type 2 diabetes) were subdivided, based on the cumulative cognition

score, together with the 30% lowest and the 30% highest participants to form a lower and a higher cognitive performance group (Supplementary Table S4.1).

(G) Finally, the 88 participants could be divided into two groups: 43 participants with a lower and 45 participants with a higher cognitive performance score, matched on age, sex, and education level.

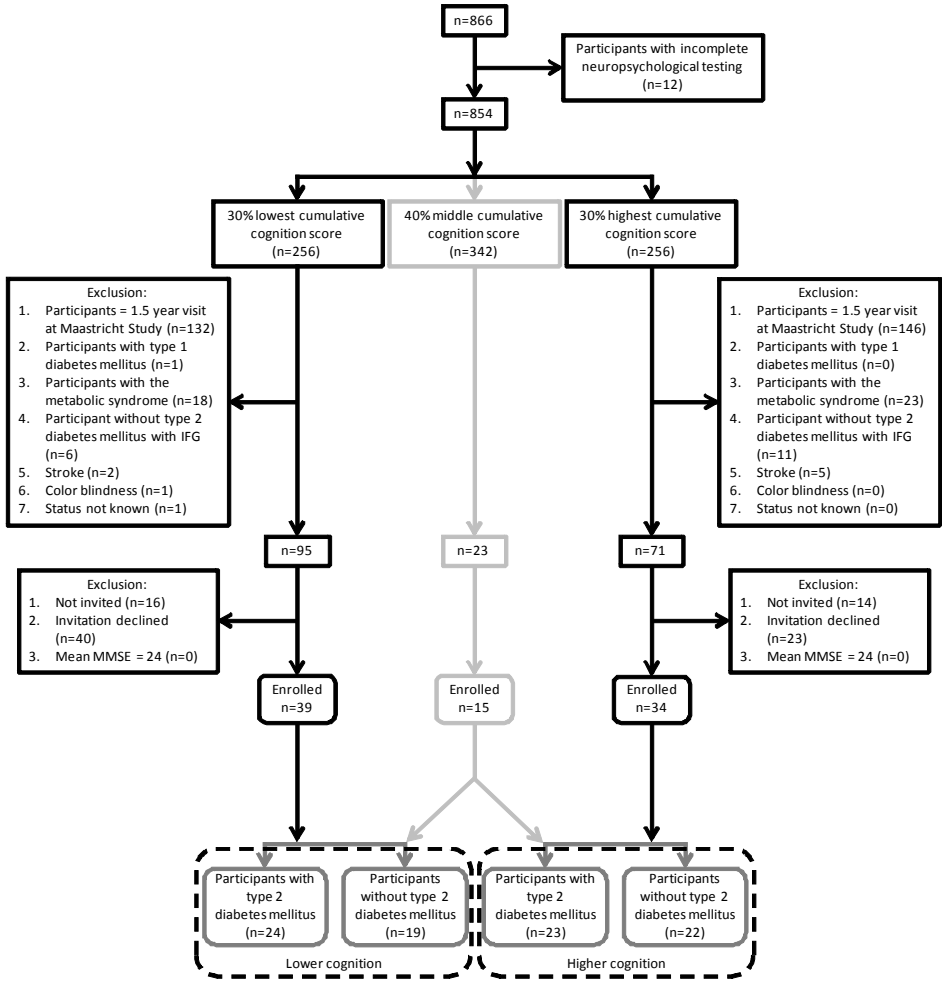

Figure S4.1 Flowchart of the selection procedure from the participants of The Maastricht Study. IFG, impaired fasting blood glucose; MMSE, Mini-Mental State Examination.

Table S4.1 Characteristics of participants in two cognition groups.

	Lower cognition (n = 43)	Higher cognition (n = 45)	p-value
Type 2 diabetes (%), n	55.8 (n = 24)	51.1 (n = 23)	0.658[a]
Age (years)	61.5 ± 9.3	63.1 ± 6.5	0.342
Sex, male (%), n	58.1 (n = 25)	57.8 (n = 26)	0.973[a]
Education			0.603[a]
Low (%), n	11.6 (n = 6)	22.2 (n = 10)	
Middle (%), n	41.9 (n = 20)	42.2 (n = 19)	
High (%), n	46.5 (n = 17)	35.6 (n = 16)	
Cognitive score			
WLT total score	36.7 ± 10.1	49.3 ± 9.0	<0.001
Stroop (sec)	63.1 ± 34.3	35.9 ± 13.3	<0.001
Verbal fluency	19.8 ± 5.2	27.3 ± 5.8	<0.001
Cumulative cognition score	-2.40 ± 2.22	2.08 ± 1.25	<0.001

Data are mean ± SD. WLT, (verbal memory) Word Learning Test. Independent samples Student *t*-test; [a]Pearson χ^2-test.

Selection procedure for image analysis

After careful analyses, data from 39 and 36 participants with type 2 diabetes (for right and left hippocampus, respectively) and 34 participants without type 2 diabetes (both hippocampi) remained suitable for final analysis, and data from 18 participants was excluded due to incomplete data (n=8), non-physiological IVIM values according to Federau et al.[1] (n=3), claustrophobia (n=2), impaired FBG levels (n=1), parkinsonism (n=1), brain injury due to an accident (n=1), an incidental finding (i.e. tumor, n=1), and a susceptibility artifact (n=1).

Data analysis

The original IVIM model describes the relationship between signal intensity (*S*) and the applied diffusion sensitization (*b*):

$$\frac{S(b)}{S_0} = (1-f)e^{-bD} + fe^{-b(D+D^*)} \qquad (1)$$

where S_0 is the signal intensity with no diffusion weighting (*b* value is 0), *f* is the perfusion fraction (relative blood volume in voxel), *D* is the diffusion coefficient of tissue water, and *D** is the pseudodiffusion coefficient of vascular water.[2] We also consider *fD** (i.e. *f* times *D**), which has previously been shown to be related to the classical cerebral blood flow (CBF).[3,4]

Incorporating inversion prepulses to minimize CSF contamination and accounting for different relaxation times between blood and gray matter, the adapted IVIM model becomes[5]:

$$\frac{S(b)}{S_0} = \frac{\begin{array}{c}(1-f)\cdot\left(1-2e^{-\frac{TI}{T_{1GM}}}+e^{-\frac{TR}{T_{1GM}}}\right)\cdot e^{-\frac{TE}{T_{2GM}}-b\cdot D}\\ +f\cdot\left(\left(1-e^{-\frac{TR}{T_{1bl}}}\right)\cdot e^{-\frac{TE}{T_{2bl}}-b\cdot(D+D^*)}\right)\end{array}}{\begin{array}{c}(1-f)\cdot e^{-\frac{TE}{T_{2GM}}}\cdot\left(1-2e^{-\frac{TI}{T_{1GM}}}+e^{-\frac{TR}{T_{1GM}}}\right)\\ +f\cdot e^{-\frac{TE}{T_{2bl}}}\cdot\left(1-e^{-\frac{TR}{T_{1bl}}}\right)\end{array}} \quad (2)$$

where TI, TR, and TE are the inversion, repetition and echo time of the IVIM sequence, respectively. T_{1GM}, T_{1bl}, T_{2GM}, and T_{2bl} are the longitudinal and transverse relaxation times of gray matter tissue and blood at 3-Tesla, for which we used 1331, 1624, 110, and 275 ms, respectively.[6-8]

The analysis was applied to the segmented gray matter of each hippocampus. Gray matter segmentation was obtained from the extraction of the hippocampus in FreeSurfer software[9], and discarding the white matter voxels. To minimize signal intensity effects of hippocampal boundaries due to potential misregistrations and to reckon with non-homogenous spatial distribution of IVIM measures (Figure 4.1, manuscript), the median rather than mean signal intensity was calculated. Subsequently, the curve of IVIM signal versus *b* value was fitted for the left and right hippocampus separately, using a two-step approach as described by Federau et al.[1], in Matlab (The Mathworks, Natick, Massachusetts). In the first step, a selection of the data points (*b* values 200-1500 s/mm^2) was fitted to a mono-exponential function (equation 3), which can be derived from equation 2, assuming that *D** is much larger than *D* and that constants independent of *b* are not of interest, to calculate *D*:

$$S(b) = S_0^{'}\, e^{-b\cdot D} \quad (3)$$

with $S_0^{'}$ constant (of non-interest, incorporating, for instance, the denominator of equation 2). In the second step, the complete set of data points was fitted to the full biexponential function (equation 2), with *D* fixed to the value obtained from the first step, to calculate f and *D**. Weighting was implemented in the fitting algorithms according to the number of signal averages per *b* value. A fitting example of the two-step approach is given in Supplementary Figure S4.2. Finally, after fitting, the IVIM measures of the two hippocampi were averaged.

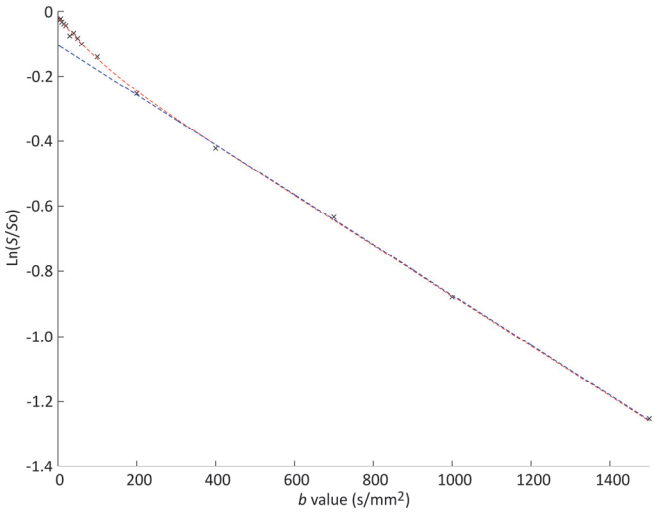

Figure S4.2 A two-step fitting example of signal decay as function of *b* value in the left hippocampus of a participant with type 2 diabetes. In the first step, the curve (dashed blue line) was fitted for *b* values ≥200 s/mm^2, yielding the diffusion coefficient *D*. In the second step, the curve was fitted using a biexponential model (dashed red line) for all *b* values, yielding perfusion fraction *f* and pseudodiffusion coefficient *D** while *D* was kept constant.

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Chapter 5

Cerebral blood flow, blood supply, and cognition in type 2 diabetes mellitus

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Abstract

We investigated whether type 2 diabetes and the presence of cognitive impairment are associated with altered cerebral blood flow. Forty-one participants with and thirty-nine without type 2 diabetes underwent 3-Tesla MRI flow scans, including a quantitative flow technique to measure (macrovascular) blood flow in the internal carotid artery and an arterial spin labeling technique to measure (microvascular) perfusion in the gray matter (GM). Three different analysis methods were used to quantify the cerebral blood flow (CBF): a region of interest analysis, an overlapping voxel-based statistical parametric mapping technique, and a 'distributed deviating voxels' method. Participants with type 2 diabetes exhibited significantly more tissue with low CBF values in the cerebral cortex and, particularly, the subcortical GM (3.8-fold increase), where the hypoperfusion was independent of atrophy. No associations were observed for CBF in other cortex regions with diabetes status, for carotid blood flow with diabetes status, or for CBF or flow in relation with cognitive function. To conclude, a novel analysis method that tallies total 'distributed deviating voxels' demonstrates hypoperfusion in participants with type 2 diabetes, particularly in the subcortical GM, which was not associated with cognitive performance. Whether a vascular mechanism underlies cognitive decrements remains therefore inconclusive.

Introduction

Type 2 diabetes mellitus is associated with cognitive decrements and an increased risk to develop dementia.[1] Furthermore, diabetes is related to complications related to damage of large blood vessels, including macrovascular disease such as coronary artery disease, peripheral vascular disease, and stroke. In addition, many complications of diabetes due to impairment of small blood vessels arise, including neuropathy, nephropathy, and retinopathy.[2] In the brain, type 2 diabetes is associated with white matter hyperintensities (WMHs), often presumed to be of vascular origin. Altered cerebral hemodynamics is one of the potential mechanisms thought to underlie the characteristic cognitive decrements.[3] Rather than studying WMHs, which are structural end-stage manifestations of impaired cerebral hemodynamics, it is also possible with advanced MRI techniques to investigate more functional or physiological cerebral characteristics, which may precede these structural changes. A prime candidate for this is actual cerebral blood flow (CBF), which can be measured noninvasively using arterial spin labeling, an MRI method that uses magnetically labeled arterial blood as a tracer.[4]

Several studies have attempted to relate type 2 diabetes with alterations in CBF, using a variety of techniques, study designs, and patient selection criteria, but results appear therefore not consistent as some report hypoperfusion, while others do not.[5] Most global CBF analysis methods either average over a volume to summarize the characteristics of that region of interest[6], or assume a certain overlap of local perfusion abnormalities over subjects using voxel-based statistical parametric mapping techniques.[7] As the effect of type 2 diabetes on CBF is likely to be subtle, the former method might not be sensitive enough to detect changes, especially when relatively large regions are analyzed. The latter method assumes a regional anatomical overlap of tissue alterations, which might not be apt for a non-focal disease such as diabetes. The current study introduces an alternative method of analysis that aims to overcome these issues. In this method, the number of voxels that statistically deviate from a normative value are recorded as 'distributed deviating voxels' and their numbers are compared between groups. In addition to CBF, which is a local measure of tissue perfusion, it is also relevant to consider the functionality of the feeding arteries. Especially the (internal) carotid arteries are of interest, as these conduit arteries provide to a large extent the blood supply to the cerebrum.

We set out to address in a non-demented population of patients with type 2 diabetes, with a range in cognitive performance, and healthy controls, whether diabetes and cognitive function are related to alterations in (macrovascular) blood flow in the internal carotid artery and (microvascular) perfusion in the cerebral gray matter (GM).

Materials and methods

Study population

Forty-seven participants with type 2 diabetes and forty-one participants without diabetes were recruited from the first 866 participants of The Maastricht Study for additional brain MRI measurements. The Maastricht Study is an ongoing observational prospective population-based cohort study, which focuses on the etiology, pathophysiology, complications and comorbidities of type 2 diabetes. Participants are between 40-75 years of age and live in the southern part of the Netherlands.[8] Participants are considered to have diabetes according to the WHO 2006 criteria if they use diabetes medication, or if they have a fasting blood glucose ≥7.0 mmol/l, and/or a 2-h blood glucose ≥11.1 mmol/l after an oral glucose tolerance test. Participants without diabetes are characterized by fasting blood glucose <6.1 mmol/l and a 2-h blood glucose <7.8 mmol/l. At baseline inclusion, participants underwent an extensive battery of measurements, including cognitive performance tasks, blood pressure measurements, and blood sampling. A detailed overview of all procedures is provided elsewhere.[8] After the baseline measurements of The Maastricht Study were completed, participants were invited to participate in this additional MRI examination.

Participants with the highest and lowest cognitive scores were selected from the first 866 participants to obtain a range in cognitive scores and to increase the probability of finding MRI effects associated with cognitive function (Table 5.1). The detailed selection procedure has been described previously.[9] In brief, the division of participants into a low and high cognition group was based on a cumulative score of three neuropsychological tests covering the domains of verbal memory, attention and flexibility, and executive functioning (Table 5.1). These scores were adjusted for age, sex, and education level using linear regression analysis.

Exclusion criteria for participants were: (A) a known history of stroke or other neurological or neuropsychiatric disease, (B) a time span between enrollment in The Maastricht Study and MRI >1.5 years, (C) incomplete cognitive assessment, (D) type 1 diabetes mellitus, (E) for participants without diabetes, an impaired fasting blood glucose level, (F) mild cognitive impairment or dementia, (G) color blindness, and (H) unknown diabetes status. The low and high cognition groups were matched for age, sex, and education level, and displayed a comparable number of participants with and without type 2 diabetes (Table 5.1).

After taken into account those who declined the invitation and exclusion of participants with MRI contraindications, a total of 47 and 41 participants with and without type 2 diabetes were included, respectively.

Prior to MRI, these participants underwent a general cognitive function test (Mini-Mental State Examination, MMSE[10]) to assess clinically significant differences in cognitive performance compared with the baseline tests at enrollment in The Maastricht Study. The study was approved by the Medical Ethics Committee of the

Maastricht University Medical Center (MUMC+), the Netherlands, and all participants gave written informed consent. The study was registered at http://www.clinicaltrials.gov with identifier NCT01705210.

Table 5.1 Characteristics of the two cognition groups.[a]

	Lower cognition (n = 40)	Higher cognition (n = 40)	p-value
Type 2 diabetes (%), n	55.0 (n = 22)	47.5 (n = 19)	0.5[b]
Age (years)	61.1 ± 9.5	62.6 ± 6.6	0.4
Sex, male (%), n	57.5 (n = 23)	55.0 (n = 22)	0.8[b]
Education			0.8[b]
Low (%), n	15.0 (n = 6)	20.0 (n = 8)	
Middle (%), n	47.5 (n = 19)	45.0 (n = 18)	
High (%), n	37.5 (n = 15)	35.0 (n = 14)	
15-WLT total score	37.1 ± 10.0	49.8 ± 9.2	<0.001
Executive functioning (sec)	63.3 ± 35.2	34.8 ± 12.7	<0.001
Verbal fluency	20.3 ± 4.9	27.3 ± 5.5	<0.001
Cumulative cognition score	-2.30 ± 2.18	2.08 ± 1.28	<0.001

Data are mean ± SD. WLT, (verbal memory) Word Learning Test. [a]only participants who were included in the final analysis; Independent samples *t*-test; [b]Pearson χ^2-test.

Magnetic resonance imaging

MRI data were acquired on a 3T scanner (Achieva TX, Philips Healthcare, Best, the Netherlands) using a 32-element head coil for parallel imaging. The MRI protocol consisted of structural scans for neuroradiological evaluation (including T1-, T2-, T2*-weighted and fluid attenuated inversion recovery sequences), phase-contrast angiograms, quantitative flow of the carotid artery, and whole cerebrum arterial spin labeling. A three-dimensional T1-weigthed (T1) fast field echo sequence (TR/TE 8.1/3.7 ms, 8° flip angle, 1 mm isotropic voxel size, 170 continuous slices, matrix size of 240 x 240) was used as anatomical reference.

Vascular anatomy from the common carotid artery to a level distal to the circle of Willis was determined using three-dimensional phase-contrast MR angiography. Maximum intensity projections in orthogonal directions resulted in three-dimensional angiograms which were used to position the two-dimensional slice for the quantitative flow estimation (Q-flow, Philips Medical Systems) (Figure 5.1A) and the labeling slice for quantitative CBF estimation (Figure 5.1B).

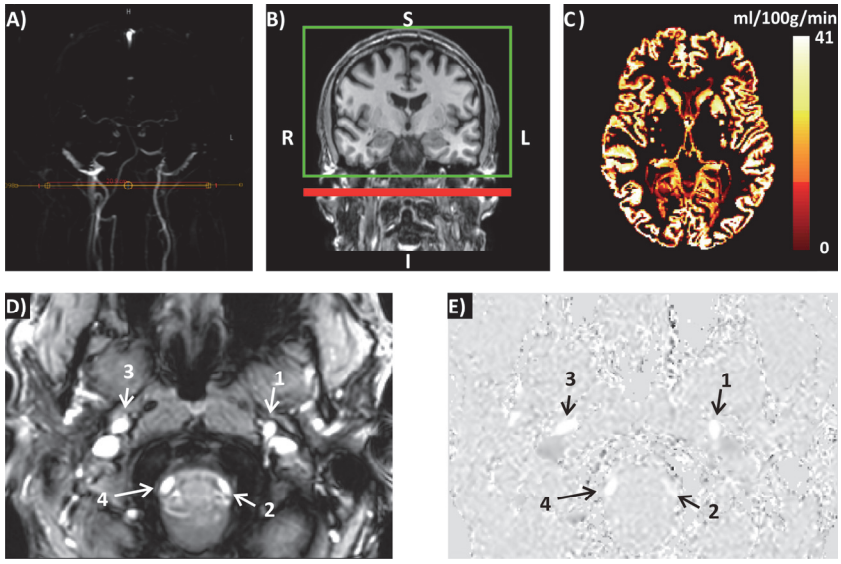

Figure 5.1 **A**) Coronal maximum intensity projections derived from phase-contrast angiography with indication of the slice for quantitative flow measurement in the internal carotid artery in a participant with type 2 diabetes. **B**) Sagittal T1 weighted image with labeling slice (red), which was positioned at the same location of the slice for quantitative flow estimation, and imaging volume (green). **C**) Resulting transverse CBF map. **D**) Magnitude and **E**) phase images of the carotid region, 1 left internal carotid artery, 2 left vertebral artery, 3 right internal carotid artery, 4 right vertebral artery.

The Q-flow technique was based on a single-slice, multiphase, fast-field echo sequence which encoded velocities parallel to the slice-encode direction. The slice was placed perpendicular to the internal carotid artery, distal to the bifurcation, on a position where the artery appeared least tortuous. Measurements were made in both the left and right sides of the carotid arteries, and were subsequently averaged over the entire cardiac cycle (Figure 5.1D). Carotid flow images were acquired using 2D fast cine PC-MRI pulse sequence with retrospective ECG gating with 15 time frames covering the entire cardiac cycle. Phase-contrast parameters were as follows: TE/TR 8/13 ms, flip angle 10°, field-of-view 150x105 mm^2, matrix 128x88, and slice thickness 6 mm. Flow direction: craniocaudal, encoding velocity: 120 cm/s.

Subsequently, a pseudo-continuous (PC)ASL 2D multislice sequence was acquired with a TR/TE of 3847/14 ms, voxel size of 3x3x7 mm^3, matrix size of 80x80x17, a post labeling delay (PLD) of 1525 ms for the first slice (PLD of last slice was 2085 ms) and a label duration of 1650 ms. The labeling slice was positioned at the same location distal to the bifurcation as for the Q-flow technique (Figure 5.1B). Slices were obtained in the feet-head direction (35 ms per slice), and 50 control-tag pairs were acquired. A single proton density (PD) sequence was acquired with the same geometric properties as the

PCASL sequence and a TR of 10 s to scale the PCASL signal intensity to an absolute CBF value.

Data analysis

The T1 weighted images were automatically segmented to the obtain total intracranial volume (ICV), and lateral ventricle size using the FreeSurfer software package (Martinos Center for Biomedical Imaging, Boston, USA).[11] The total lateral ventricular volume relative to ICV was taken as a measure for atrophy.

Carotid flow images were analyzed in Matlab. Arteries were visually identified and delineated on the magnitude image to segment the flow regions on the phase images for all time frames. Flow was calculated in cm^3/s by integrating the velocity value over the pixels of the vessel cross-section and averaging over the time frames. Due to technical difficulties, not all flow measurements were successful. For the final flow analysis, reliable data were available in 36 (of 47) participants with type 2 diabetes and 37 (of 41) healthy controls.

For ASL, motion correction was performed relative to the mean of the control images with FMRIB's linear image registration tool (FLIRT) using a mutual-information algorithm.[12] Next, the label images were subtracted from the control images. Control-tag pairs were removed when visual inspection of the subtraction result showed abnormal results. CBF maps calculation (Figure 5.1C) was based on an adaptation of the formula proposed in the ASL whitepaper presented by the ISMRM Perfusion Study Group and the European Consortium for ASL in Dementia[13] with addition of correction for a 2D multislice acquisition scheme and a correction factor for background suppression[14]:

$$\mathrm{CBF} = \frac{6000 \cdot \lambda \cdot (\mathrm{SI}_{\mathrm{control}} - \mathrm{SI}_{\mathrm{label}}) \cdot e^{\frac{T_{\mathrm{delay}} + T_{\mathrm{slice}}\,(z-1)}{T_{1,\mathrm{blood}}}}}{2 \cdot \alpha \cdot \alpha_{\mathrm{inv}} \cdot T_{1,\mathrm{blood}} \cdot \mathrm{SI}_{\mathrm{PD}} \cdot \left(1 - e^{\frac{-\tau}{T_{1,\mathrm{blood}}}}\right)}$$

where λ is the blood-brain partition coefficient (set at 0.9 ml/g), $SI_{control}$ and SI_{label} are the means over time of the control and label images, respectively, T_{delay} is the post label delay (1525 ms), T_{slice} is the acquisition time for a single slice (35 ms), z is the slice number, $T_{1,blood}$ is the longitudinal relaxation time of blood (set at 1650 ms for 3T), α is the labeling efficiency (set at 0.85), α_{inv} is a correction factor for the background suppression (set at 0.83), SI_{PD} is the signal intensity of the proton density image and τ is the label duration (1650 ms). To account for partial volume effects at GM- white matter (WM) borders, a GM probability map was created from the T1-weighted structural scan using FAST (FMRIB's automated segmentation tool[15]). This allowed for correction of the CBF for the amount of GM in a voxel, by dividing each voxel by its GM probability, with a minimum probability threshold of 50%.[16] Finally, CBF maps were coregistered to

Montreal Neurological Institute space using the FNIRT routine in FSL[17] in order to facilitate the use of the MINC1 atlas.[18]

For the regional analysis, CBF values were expressed in ml/100g/min and averaged over the GM of the following regions: whole cerebral cortex, frontal, temporal, parietal, and occipital cortex, and subcortical GM (i.e. accumbens, caudate, pallidum, putamen, and thalamus), as defined by the International Consortium for Brain Mapping 2009c nonlinear symmetric MINC1 atlas.[18]

Additionally, a voxel-based statistical parametric mapping analysis was performed using routines from the SPM8 software package (Wellcome Department of Cognitive Neurology). Age and sex were added as covariates, and correction for multiple comparisons was applied using a False Discovery Rate of 5%.

Finally, for the 'distributed deviating voxels' analysis, the CBF maps of the subjects were transformed on a pixel-by-pixel basis into a statistical *z*-score (defined as $[(x_i - x_{ref})/SD]$) maps using the averaged CBF values of the controls, with the highest cognitive performance (x_{ref}), as reference.[19] The *z*-score maps of the participants with the highest cognitive performance within the control group were based on the values of the other high cognitive performance controls (n-1). For all regions (whole brain, frontal, temporal, parietal, and occipital cortex, and subcortical GM, as defined by the MINC1 atlas), the voxels were counted that deviated with 99% confidence, corresponding to *z*-score of $z_{\alpha/2} = 2.576$. Both positive and negative *z*-values were considered separately. The number of voxels are reported as percentage of the total intracranial volume.

For the CBF analysis, reliable data were available in 41 (of 47) participants with type 2 diabetes and 39 (of 41) healthy controls.

Statistical analysis

Descriptive participants' characteristics are reported as mean ± standard deviation. Group characteristics were tested by use of independent samples *t*-tests and Pearson χ^2-tests with SPSS (Statistical Package for Social Sciences, version 20, IBM Corp., Armonk, NY, USA), with α=0.05.

Differences in carotid flow and CBF measures between diabetes and controls were also tested by use of independent samples *t*-tests. When differences were significant, they were subsequently explored with linear regression analysis, to correct for differences in clinical characteristics between groups. For carotid flow, the linear regression analysis was adjusted for age and sex. For CBF measures (global GM CBF, and number of deviating CBF voxels), first age and sex were used as covariates in the analysis. Subsequently, relative lateral ventricular volume (as measure for atrophy), and carotid flow were separately added as covariates. Atrophy was included, as it is known to affect CBF.[20] Furthermore, to limit the number of statistical tests for the CBF analyses, in a staged approach, first only the whole cerebrum was considered. When significant differences were observed for the whole cerebrum, post-hoc tests were

subsequently performed to evaluate the sub-regions (frontal, temporal, parietal, and occipital cortex, and subcortical GM).

Finally, to evaluate the effect of cognitive performance, a dichotomous value (low versus high cognition) was added to the linear regression models for flow and CBF.

Results

Table 5.1 shows the baseline characteristics of the low and high cognitive performance groups, as participants were selected based on cognitive status. The groups were matched for age, sex, education and diabetes status. Table 5.2 lists the clinical characteristics of the participants, based on diabetes status. Type 2 diabetes was associated with higher fasting blood glucose levels, higher HbA_{1c} levels, higher body mass index, higher diastolic as well as systolic blood pressure, more often hypertension, and higher WM lesion loads (Table 5.2). With respect to cognition, participants with type 2 diabetes scored significantly lower on baseline MMSE score (p=0.006) compared with participants without diabetes. Baseline MMSE did not differ from repeated MMSE (p=0.3).

Table 5.2 Clinical characteristics of participants with and without type 2 diabetes.

	Participants with type 2 diabetes (n = 41)	Participants without type 2 diabetes (n = 39)	p-value
Type 2 diabetes-related variables			
Duration of diabetes (years)	9.8 ± 6.7	-	
Fasting blood glucose (mmol/l)	7.5 ± 1.2	5.1 ± 0.3	<0.001
HbA_{1c} (%)	6.7 ± 0.4	5.6 ± 0.4	<0.001
HbA_{1c} (mmol/mol)	50.2 ± 4.9	38.0 ± 4.5	<0.001
Type 2 diabetes medication			
None (%)	12.2	100	<0.001[a]
Insulin (%)	2.4	-	
Oral medication (%)	75.6	-	
Insulin and oral medication (%)	9.8	-	
Clinical variables			
BMI (kg/m^2)	29.2 ± 3.5	24.7 ± 2.8	<0.001
SBP (mmHg)	152 ± 18	131 ± 18	<0.001
DBP (mmHg)	83 ± 10	76 ± 13	0.013
Cardiovascular disease (%)	20.5	13.5	0.4
Hypertension (%)	95.1	38.5	<0.001[a]
Smoking status, never/former/current (%)	23.7/71.1/5.3	23.7/55.3/21.1	0.114[a]
Cognitive score			
Cumulative cognition score	-0.60 ± 3.17	0.40 ± 2.36	0.117
Baseline MMSE total score	28.6 ± 1.4	29.4 ± 0.8	0.006

Data are mean ± SD. HbA_{1c}, glycated hemoglobin; BMI, body mass index, SBP, systolic blood pressure; DBP, diastolic blood pressure; MMSE, Mini-Mental State Examination. Independent samples *t*-test; [a]Pearson χ^2-test.

The flow analysis revealed that the flow in the internal carotid arteries in type 2 diabetes (10.5±2.2 cm^3/s) was not significantly different from controls (10.8±1.8 cm^3/s, p=0.6). For global measurements of GM CBF, significantly lower values were found in diabetes (28.3±5.6 ml/100g/min) compared with controls (31.5±5.9 ml/100g/min, p=0.014). However, after including age and sex as covariates, the difference disappeared (p=0.5), also after adding atrophy (p=0.7) and carotid flow in a separate analysis (p=0.5). Therefore, no post-hoc analyses for sub-regions were performed. Additionally, the statistical parametric mapping CBF technique also did not reveal any significant locally overlapping differences.

The 'distributed deviating voxels' method revealed approximately twice as many negatively deviating (low flow) voxels in the whole cerebrum for type 2 diabetes (0.10±0.08% of ICV) compared with controls (0.05±0.03% of ICV, p<0.001). Therefore post-hoc analyses were performed for the sub-regions (see Table 5.3). This analysis revealed that there were significantly more negatively deviating voxels for participants with type 2 diabetes in frontal, temporal, parietal, and subcortical GM regions (p<0.002), which only remained significant after adjusting for atrophy in the subcortical GM (3.8-fold increase, p=0.042, Figure 5.2). A similar trend was found for the occipital region (p=0.095). No regions were found showing positively deviating (high flow) voxels (p>0.35). Correcting CBF measures for carotid flow did not affect the results. Furthermore, linear regression, with inclusion of cognitive performance as covariate, also did not yield any significant association between cognition with flow or CBF (p>0.5).

Table 5.3 Fraction of negative 'deviating voxels' with low flow (hypoperfusion) in GM, relative to intracranial volume.

	Participants with type 2 diabetes (n = 41)	Participants without type 2 diabetes (n = 39)	p-value[a]
Cerebral cortex	0.10 ± 0.08%	0.05 ± 0.03%	<0.001
Frontal cortex	0.04 ± 0.03%	0.02 ± 0.01%	0.002
Temporal cortex	0.03 ± 0.02%	0.01 ± 0.01%	<0.001
Occipital cortex	0.00 ± 0.00%	0.00 ± 0.00%	0.095
Parietal cortex	0.01 ± 0.01%	0.00 ± 0.00%	<0.001
Subcortical GM	0.02 ± 0.02%	0.01 ± 0.01%	<0.001[b]

Data are mean ± SD. GM, gray matter. [a]Independent samples *t*-test, [b]Significant after correcting for age, sex, and atrophy.

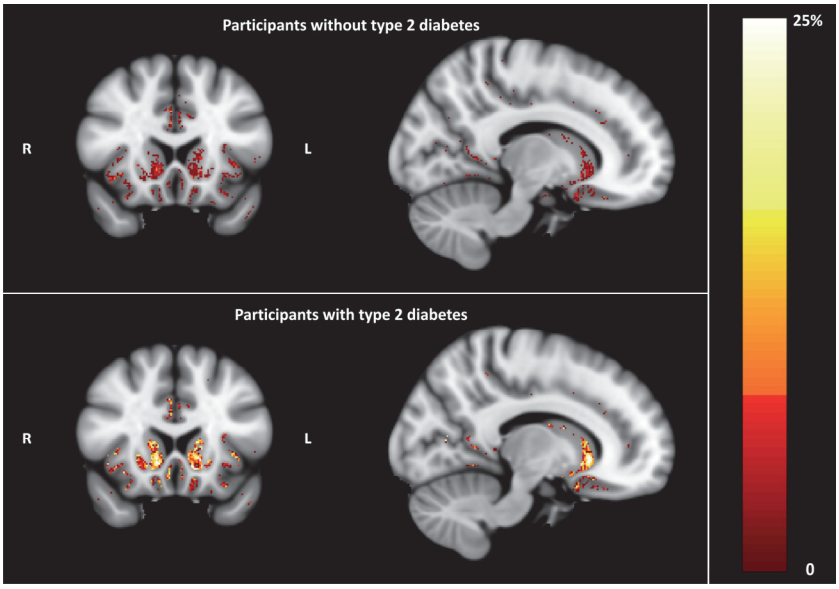

Figure 5.2 Normalized T1 weighted images, with as overlay the percentage of participants within a group displaying negatively 'deviating voxels' (indicative of hypoperfusion) for participants without type 2 diabetes (**upper figure**) and participants with type 2 diabetes (**lower figure**). Note the high percentage (indicating hypoperfusion) for participants with type 2 diabetes in the subcortical GM, especially in the nucleus accumbens and caudate structures.

Discussion

This study was performed to investigate whether type 2 diabetes and cognitive impairment are associated with differences in cerebral blood flow. To this end, participants with type 2 diabetes, with a range in cognitive performance, and healthy controls, were investigated by use of MRI flow techniques: a macrovascular flow technique to study the blood supply from the internal carotid artery, and an arterial spin labeling technique to measure the microvascular GM perfusion in terms of CBF. Participants with diabetes exhibited significantly more GM tissue with low CBF values in the cerebral cortex and, particularly, the subcortical GM. No (independent) associations were found between carotid artery or GM blood flow and cognitive decrements.

Cerebral hemodynamics

Type 2 diabetes was not associated with carotid artery blood flow, which is fully in agreement with previous studies[21,22], and indicates that blood supply to the brain is not affected in the investigated diabetes population. However, the current study did

observe more GM tissue with abnormally low flow values in the cortex and the subcortical GM in participants with type 2 diabetes, which can be interpreted as evidence for cerebral hypoperfusion. This finding concurs with the hypothesis that type 2 diabetes is associated with impaired cerebral hemodynamics, a mechanism that might in part underlie the cognitive decrements or accelerated cognitive aging associated with type 2 diabetes.[3] It has been suggested that type 2 diabetes can affect the glucose and insulin transfer across the blood-brain barrier, hence altering regional metabolism and microcirculation.[23] Chronic hyperglycemia has been shown to decrease regional blood flow and increase membrane permeability, eventually prompting permanent brain cell damage.[24] A progressive metabolic disturbance in the cerebrovascular bed seems to disturb blood flow and accelerate WM degeneration.[23]

An explanation why the CBF association was detected in the subcortical GM, but not in the cortical regions, might be a different local microvascular architecture: vessel density is lower and vessels are more deep than collateral in the subcortical region compared with the cortical region.[25] Furthermore, in small vessel disease, for which type 2 diabetes is a risk factor, microbleeds are more often found subcortical[26], indicating that the subcortical region might have a higher susceptibility to vascular pathology (e.g. ischemia or hypoperfusion).

Over the last 2 decades, the association of type 2 diabetes with CBF has been investigated in a number of studies, using various techniques including single-photon emission computed tomography, positron emission tomography and ASL. Although some report hypoperfusion in diabetes[23,27,28], other studies did not find any association with CBF.[21,29,30] It has been shown that most studies reporting on hypoperfusion in type 2 diabetes typically use small populations, include patients with severe complications, and did not account for atrophy, which has been shown to largely explain hypoperfusion.[20] The latter notion was also evidenced in the current study, as including atrophy as covariate decreased the number of regions with hypoperfusion, but the results in subcortical GM remained significant. Larger, epidemiologic studies have failed so far to find associations of CBF with type 2 diabetes.[6,31]

Tiehuis et al.[21] suggested that despite the absence of an association of type 2 diabetes with CBF under resting conditions, it is still possible that diabetes is associated with altered cerebrovascular reactivity. Indeed, research specifically designed to assess cerebral vasoreactivity using ASL under hypercapnic conditions reported that patients with type 2 diabetes exhibit diminished global and regional cerebral vasoreactivity.[30,32] In addition, the work from Duarte et al.[33], who used tasked-based functional MRI, showed that type 2 diabetes is associated with impaired neurovascular coupling, as the hemodynamic response function is different from healthy controls.

Perfusion quantification

A possible explanation for the fact that the current study does find evidence of cerebral hypoperfusion under resting conditions, even when correcting for atrophy, might be

attributable to the potentially higher sensitivity of the analysis applied. The current study applied three distinctly different methods to assess the effect of type 2 diabetes or cognitive status on CBF. The applied methods were sensitive to either 1) global effects (region of interest analysis), 2) locally overlapping focal effects (voxel-based statistical parametric mapping), or 3) more spatially distributed (diffuse) effects ('deviating voxels'). Only the deviating voxel method appeared sensitive enough to detect a significant effect of diabetes on CBF.

An explanation for this is that the CBF effect of diabetes is probable subtle (which is reflected by the very low percentage of deviating voxels), and not necessarily localized at identical spots across different individuals. These effects might not be picked up by a region of interest analysis, as subtle effects in sub regions might be overshadowed by noise from other sub regions when taking an average over a selection of sub regions. Furthermore, when a large number of regions is considered, one has to correct for multiple comparisons, thereby decreasing the likelihood of obtaining significant effects. Additionally, voxel-based statistical parametric mapping technique could be insensitive to such effects, as this method assumes a certain regional overlap across individuals of altered tissue. In contrast with more focal pathologies such as epilepsy and stroke, diabetes is a systemic disease, and although there might be regional differences, there is no evidence that these regions should be identical for different patients with type 2 diabetes. The current study introduced an alternative method of analyzing ('distributed deviating voxels') that proved to be more sensitive than the other two analysis techniques. By tallying the number of deviating voxels, changes in CBF can be detected that are subtle while not requiring overlap at the exact location of the hemodynamic disturbance over the studied participants.

Association with cognition

In contrast with other studies on blood flow in type 2 diabetes[3,21,29-31], no significant associations of cognitive performance status were found with CBF. In the current, non-demented population of participants with type 2 diabetes, the cognitive performance for all participants either with or without diabetes falls within the range considered cognitively normal (i.e. MMSE>27). The individuals that do experience cognitive decrements still exhibit substantially better cognitive performance scores than for example patients who are suspect of a cognitive disorder (MMSE<25). A potential implication of this study that found a significant association between CBF and type 2 diabetes status, but not between CBF and cognitive performance, is that diabetes-induced cerebrovascular alterations potentially precede the cognitive decrements. Therefore, an altered CBF might be a potential biomarker to identify patients at risk of developing cognitive problems.

Clinical implications

Type 2 diabetes was found to be associated with hypoperfusion, hence the results of this study indicate that treatment to avoid decline of or even improve vascular function (e.g. with antihypertensive or antiplatelet drugs) could be beneficial in patients with diabetes. Future studies that further elucidate these biological alterations might reveal new opportunities to monitor therapeutic/lifestyle interventions for improving cognition and/or prevention of cognitive impairment. A longitudinal set up is required to investigate whether type 2 diabetic patients with cerebral hypoperfusion are at increased risk for developing cognitive decrements in the near future.

Strengths and limitations

The strengths of the present study comprise: first, the extensive (cardiovascular) characterization of the participants. Second, the investigation included both microvascular GM perfusion and macrovascular blood supply, and incorporated a variety of CBF analysis methods. Third, the quantified measures for carotid flow (approximately 11 cm^3/s) and CBF (approximately 32 ml/100g/min) are comparable with previously reported values of 8 cm^3/s for carotid flow[34] and 30±5 ml/100g/min ($[^{15}O]H_2O$ PET) and 34±5 ml/100g/min (ASL) for CBF[35], which is indicative of sound quantitative results. On the other hand, limitations should also be considered. A first limitation is the cross-sectional design of the study. Nevertheless, these first cross-sectional results are promising and pave the way for future (longitudinal) studies. Second, the inclusion of relative healthy participants with type 2 diabetes decreased the likelihood of finding a possible association between cognition and CBF, as observed in other studies, but might provide a more representative view of early effects of diabetes on cognition. Nonetheless, it will be interesting to include more severe diabetes participants in the future.

Conclusion

A novel analysis method that tallies total ‘deviating voxels’ demonstrates distributed hypoperfusion in type 2 diabetes, especially in the subcortical regions, whereas more traditional analysis methods appeared to be not sensitive enough. Whether a vascular mechanism underlies the cognitive decrements in type 2 diabetes remains inconclusive.

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Chapter 6

Altered GABA concentrations in type 2 diabetes mellitus are related to lower cognitive functioning

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Abstract

Type 2 diabetes mellitus is associated with accelerated cognitive decline. The underlying pathophysiological mechanisms still remain to be elucidated although it is known that insulin signaling modulates neurotransmitter activity, including inhibitory γ-aminobutyric acid (GABA) and excitatory glutamate (Glu) receptors. Therefore, we examined whether levels of GABA and Glu are related to diabetes status and cognitive performance. Forty-one participants with type 2 diabetes and thirty-nine participants without type 2 diabetes underwent detailed cognitive assessments and 3-Tesla proton MR spectroscopy. The associations of neurotransmitters with type 2 diabetes and cognitive performance were examined using multivariate regression analyses controlling for age, sex, education, BMI, and percentage gray/white matter ratio in spectroscopic voxel. Analysis revealed higher GABA+ levels in participants with type 2 diabetes, in participants with higher fasting blood glucose levels and in participants with higher HbA_{1c} levels, and higher GABA+ levels in participants with both high HbA_{1c} levels and less cognitive performance. To conclude, participants with type 2 diabetes have alterations in the GABAergic neurotransmitter system, which are related to lower cognitive functioning, and hint at the involvement of an underlying metabolic mechanism.

Introduction

Type 2 diabetes mellitus is an endocrine disorder characterized by attenuated insulin signaling and decreased cellular responsiveness to insulin. Since systemic insulin resistance is accompanied by central insulin resistance, the complications of diabetes not only involve peripheral tissues, but also the central nervous system (CNS).[1,2] Indeed, type 2 diabetes is associated with cognitive deficits[3], accelerated cognitive decline[4], and an increased risk for developing dementia and Alzheimer's disease.[4-7] Insulin signaling plays an important role in synaptic plasticity by modulating neurotransmitter channel activity, including excitatory and inhibitory receptors such as γ-aminobutyric acid (GABA) and glutamate (Glu) receptors.[8,9] Therefore, defects in brain insulin signaling may give rise to neuronal dysfunction and impaired cognitive performance.[10,11]

Proton MR spectroscopy (^{1}H-MRS) provides the unique opportunity to assess noninvasively the concentrations of neurometabolites including neurotransmitters GABA and Glu *in vivo*, through the identification and quantification of spectral peaks. GABA is the major inhibitory neurotransmitter, whereas Glu is the major excitatory neurotransmitter. At clinical field strengths (≤3.0T), GABA and Glu are difficult to quantify due to the spectral overlap with the signals of other metabolites, including *n*-acetyl aspartate (NAA), total creatine (tCr), and glutamine (Gln). However, advanced spectral editing methods have been developed that enable the detection of metabolites with strong spectral overlap. For example, the MEscher-GArwood-point resolved spectroscopy sequence (MEGA-PRESS) edited ^{1}H-MRS method can be used for the quantification of GABA.[12]

As ^{1}H-MRS facilitates the assessment of neurotransmitters GABA and Glu, it is a suitable technique to explore whether an altered neurotransmitter metabolism underlies cognitive problems in type 2 diabetes. A previous ^{1}H-MRS study with respect to neurotransmitters in depressed type 2 diabetes have reported decreased Glu levels[13] compared with healthy controls. Moreover, lower GABA levels were observed in patients with diabetic neuropathy.[14]

To our knowledge, no studies explored the neurotransmitter metabolism of impaired cognitive performance in type 2 diabetes. Therefore, the current study was designed to assess whether neurotransmitters (GABA and Glu) are related to type 2 diabetes status, cognitive performance, and the potential interaction between type 2 diabetes and cognitive performance.

Materials and methods

Study population

Forty-seven participants with type 2 diabetes and forty-one participants without type 2 diabetes were recruited from the first 866 participants of The Maastricht Study for additional brain MRI measurements. The Maastricht Study is an ongoing observational, prospective, population-based cohort study that focuses on the etiology, pathophysiology, complications, and comorbidities of type 2 diabetes. Participants are between 40-75 years of age and live in the southern part of the Netherlands.[15] Participants are considered to have diabetes according to the WHO 2006 criteria if they use diabetes medication or if they have a fasting blood glucose ≥7.0 mmol/l or a 2-h blood glucose ≥11.1 mmol/l. Participants without type 2 diabetes are characterized by fasting blood glucose <6.1 mmol/l and a 2-h blood glucose <7.8 mmol/l. At baseline inclusion, participants underwent an extensive battery of measurements, including cognitive performance tasks, blood pressure measurements, and blood sampling. A detailed overview is provided in Schram et al..[15] After their baseline measurements of The Maastricht Study, participants were invited to participate in this MRI study.

Participants with the highest and lowest cognitive scores were selected from the first 866 participants to increase the probability of finding MRI differences associated with cognitive decrements (Table 6.1). A detailed selection procedure is provided in van Bussel et al..[16] In brief, the division of participants in a low and high cognition group was based on a cumulative score of three neuropsychological tests covering the domains of verbal memory, attention and flexibility, and executive functioning (Table 6.1). For matching, the scores for each neuropsychological test were adjusted for age, sex, and education level by linear regression and the cumulative cognition score was calculated by adding the corresponding *z*-scores (standardized residuals) of the three neuropsychological tests. Exclusion criteria for participants were: (A) a known history of stroke or neurological disease, (B) if the time span between enrollment in The Maastricht Study and MRI was >1.5 years, (C) incomplete cognitive assessments, (D) type 1 diabetes mellitus, (E) for participants without type 2 diabetes, an impaired fasting blood glucose level, (F) mild cognitive impairment, (G) participants with the metabolic syndrome, (H) participants with color blindness, and (I) participants with an unknown diabetes status. The low and high cognition groups were matched on age, sex, and education level, and display a similar distribution of participants with and without type 2 diabetes (Table 6.1).

After taking into account those who declined the invitation and exclusion of participants with MRI contraindications, a total of forty-seven and forty-one participants with and without type 2 diabetes were included, respectively.

Prior to MRI, all participants underwent a general cognitive function test (Mini-Mental State Examination, MMSE[17]) to assess clinically significant differences in cognitive performance compared with the baseline cognitive tests at enrollment in The

Maastricht Study. None of the participants were excluded based on a MMSE score of ≤24. Structural brain and MR spectroscopy scans were obtained from all participants. This study was approved by the Medical Ethics Committee of the Maastricht University Medical Center (MUMC+), the Netherlands, and all participants gave written informed consent. The study is registered at http://www.clinicaltrials.gov with identifier NCT01705210.

Table 6.1 Characteristics of the two cognition groups.[a]

	Low cognitive function (n = 39)	High cognitive function (n = 41)	p-value
Type 2 diabetes (%), n	53.8 (n = 21)	48.8 (n = 20)	0.650[b]
Age (years)	61.1 ± 9.7	62.7 ± 6.6	0.369
Sex, male (%), n	56.4 (n = 22)	56.1 (n = 23)	0.978[b]
Education			0.889[b]
Low (%), n	15.4 (n = 6)	19.5 (n = 8)	
Middle (%), n	46.2 (n = 18)	43.9 (n = 18)	
High (%), n	38.5 (n = 15)	36.6 (n = 15)	
Verbal memory	37.1 ± 10.1	49.6 ± 9.1	<0.001
Executive function (sec)	63.3 ± 35.6	34.7 ± 12.6	<0.001
Verbal fluency	20.3 ± 4.9	27.6 ± 5.7	<0.001
Cumulative cognition score	-2.31 ± 2.20	2.12 ± 1.28	<0.001

Data are mean ± SD. [a]only participants who were included in the final analysis; Independent samples *t*-test; [b]Pearson χ^2-test.

Magnetic resonance imaging

MRI data were acquired on a 3T scanner (Achieva TX, Philips Healthcare, Best, the Netherlands) using a 32-element head coil for parallel imaging. The MRI protocol consisted of structural scans for neuroradiological evaluation (including T1-, T2-, T2*-weighted and fluid attenuated inversion recovery (FLAIR) sequences) and ^{1}H-MRS scans. A three-dimensional T1-weigthed (T1) fast field echo sequence (TR/TE 8.1/3.7 ms, 1.00 mm isotropic voxel size, 170 continuous slices, matrix size of 240 x 240, and 7:56 min acquisition time) was acquired and used for the positioning of the spectroscopic voxel and voxel segmentation. ^{1}H-MRS were acquired from a 3x3x3 cm^3 voxel located in the occipital lobe (Figure 6.1AB) due to its favorable signal to noise profile using a single voxel PRESS sequence (TR/TE 2000/38 ms, 128 averages, MOIST water suppression, and 4:52 min acquisition time). Additionally, a spectrum (16 averages) was recorded of unsuppressed water. For GABA, a MEGA-PRESS sequence (TR/TE 2000/68 ms, 320 averages, editing pulses at 1.9 (ON) and 7.46 ppm (OFF) interleaved in 40 blocks, MOIST water suppression, and 10:40 min acquisition time) was acquired.[12]

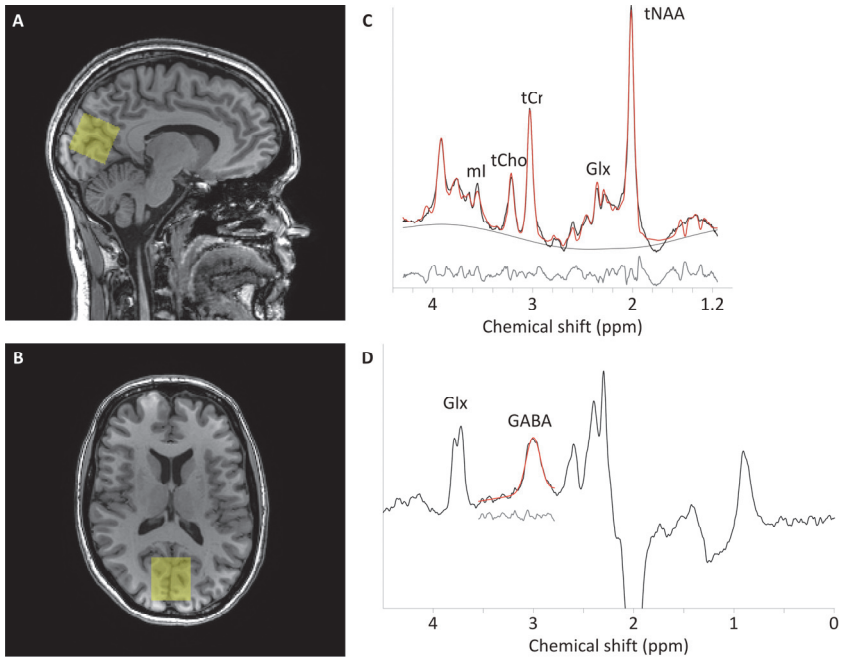

Figure 6.1 (**A**) Sagittal and (**B**) axial view of T1-weighted image of participant without diabetes indicating the ^{1}H-MRS voxel (yellow) in the occipital lobe. (**C**) Representative PRESS spectrum (black line) and LCModel fit (red line). The smooth black line shows the estimated baselines by LCModel and the gray line shows the residuals between the raw data (black line) and the fit (red line). The peaks in the spectrum represent mI, tCho, tCr, Glx, and tNAA metabolites. (**D**) Representative MEGA-PRESS spectrum (black line) and the Gannet fit (red line), yielding GABA+ concentration. The gray line shows the residuals between the raw data and the fit.

Data analysis

The metabolite concentrations within the PRESS voxel were analyzed using the LCModel (linear combination of model spectra) software package (version 6.3-1B), which analyzes the *in vivo* MR spectra as a linear combination of the spectra of the individual metabolites.[18] LCModel performs water-scaling automatically and uses a simulated basis set. The simulated basis set was provided by dr. Provencher and included the following 16 metabolites: alanine, aspartate, creatine (Cr), GABA, glucose, Gln, Glu, glycerophosphocholine (GPC), phosphocholine (PCh), myo-inositol (mI), lactate, NAA, *n*-acetylaspartylglutamate (NAAG), scyllo-inositol, taurine, and guanine. Metabolite estimates were excluded from analysis if the Cramér-Rao lower bound, an estimate of the error in metabolite quantification and used as reliability indicator, was greater than 15% range. Glu satisfies the requirements of the Cramér-Rao lower bound <15%. Post-hoc analyses were performed for all other metabolites which fulfilled the Cramér-Rao criteria: Glx (the combined signal of Glu and Gln), mI, total choline (tCho;

sum of GPC and PCh), total NAA (tNAA; sum of NAA and NAAG), and tCr (sum of Cr and phosphocreatine). Figure 6.1C shows a typical spectrum and its best-fit model. In this manuscript, metabolic concentrations are reported relative to water[19] and expressed in institutional units (i.u.). To this end, after the *in vivo* measurement, the signal from unsuppressed tissue water was recorded from the same voxel, which served as an endogenous concentration reference. No corrections for relaxation were performed.

The GABA concentration (quantified in i.u. relative to the unsuppressed water signal from the same volume) was estimated by analyzing the MEGA-PRESS spectrum using the Gannet 2.0 toolkit, a Matlab-based quantification batch analysis tool for analyzing GABA MEGA-PRESS spectra.[12] A detailed overview is provided in Edden et al..[12] As the GABA concentration estimation likely contains contribution from macromolecules and homocarnosine, it will therefore be referred to as GABA+. Figure 6.1D shows the fitted GABA+ signal. From here on, the GABA+/H_2O ratio is referred to as GABA+ level.

The T1 images were used for the ^{1}H-MRS voxel segmentation (Figure 6.1AB) to account for differences in tissue composition which may influence the metabolite concentrations. The ^{1}H-MRS voxel was co-registered to the T1 image and automatically segmented as white matter (WM), gray matter (GM), or cerebrospinal fluid (CSF) using FSL FAST (FMRIB's Automated Segmentation Tool, Oxford University, Oxford, UK).[20] Then, percentages of WM, GM, and CSF in the ^{1}H-MRS voxel were calculated and the metabolite concentrations were corrected for CSF content. Furthermore, the ratio of GM to WM in the voxel was used as a covariate in linear regression analyses.[21]

After careful analyses, data from forty-one type 2 diabetes participants and thirty-nine participants without type 2 diabetes remained suitable for final analysis, as data from eight participants were excluded due to claustrophobia (n=2), impaired fasting blood glucose levels (n=2), parkinsonism (n=1), brain injury due to an accident (n=1), an incidental finding (i.e. tumor, n=1), and unreliable data (n=1). In addition, one included participant had a missing GABA measurement.

Statistical analysis

Descriptive participant characteristics are reported as mean ± standard deviation. Group characteristics were tested using independent samples *t*-tests and Pearson χ^2-tests using SPSS (Statistical Package for Social Sciences, version 20, IBM Corp., Armonk, NY, USA).

Linear regression analyses, adjusted for age, sex, education level, BMI[22], and percentage GM/WM ratio were performed to assess the association of the neurotransmitter concentrations (GABA+ and Glu) with type 2 diabetes status and cognition status. Furthermore, the interaction term between type 2 diabetes and cognition status (lower versus higher cognitive performance) was added to the linear regression model to investigate the combined effect of type 2 diabetes and less cognitive performance on metabolite concentrations.

All analyses were repeated replacing the dichotomous type 2 diabetes status by either fasting blood glucose levels or glycated hemoglobin (HbA_{1c}), a measure for long-term blood glucose control. In addition, post-hoc analyses were also performed for other metabolite concentrations (Glx, mI, tCho, tNAA, and tCr).

Results

Characteristics

Table 6.1 shows the baseline characteristics of the low and high cognition groups, as participants were selected based on cognitive performance. The groups were matched on age, sex, education, and type 2 diabetes status, but score different on cognition. Table 6.2 shows the clinical characteristics of participants based on diabetes status. As expected, participants with type 2 diabetes had higher fasting blood glucose levels, higher HbA_{1c} levels, higher BMI, and both higher systolic and diastolic blood pressure (Table 6.2). Participants with type 2 diabetes scored significantly worse on baseline MMSE score (p=0.006). Baseline and repeated MMSE did not differ for all participants (p=0.280).

Table 6.2 Clinical characteristics of participants with and without type 2 diabetes.

	Participants with type 2 diabetes (n = 41)	Participants without type 2 diabetes (n = 39)	p-value
Type 2 diabetes-related variables			
Duration of diabetes (years)	9.6 ± 6.7	-	
Fasting blood glucose (mmol/l)	7.7 ± 1.6	5.1 ± 0.3	<0.001
HbA_{1c} (%)	6.8 ± 0.5	5.6 ± 0.4	<0.001
HbA_{1c} (mmol/mol)	50.7 ± 5.6	38.0 ± 4.5	<0.001
Type 2 diabetes medication			
None (%)	12.2	100	<0.001[a]
Insulin (%)	2.4	-	
Oral medication (%)	75.6	-	
Insulin and oral medication (%)	9.8	-	
Clinical variables			
BMI (kg/m^2)	29.2 ± 3.5	24.7 ± 2.8	<0.001
SBP (mmHg)	152 ± 18	131 ± 18	<0.001
DBP (mmHg)	83 ± 10	76 ± 13	0.012
Cognitive score			
Baseline MMSE total score	28.6 ± 1.4	29.4 ± 0.8	0.006
Repeated MMSE total score	28.7 ± 1.2	29.1 ± 0.9	0.169

Data are mean ± SD. HbA_{1c}, glycated hemoglobin; BMI, body mass index, SBP, systolic blood pressure; DBP, diastolic blood pressure; (baseline / repeated) MMSE, (The Maastricht Study / before MRI) Mini-Mental State Examination. Independent samples *t*-test; [a]Pearson χ^2-test.

Table 6.3 Relationship between the neurotransmitters GABA+ and Glu with type 2 diabetes (either dichotomous status, fasting blood glucose levels, or HbA_{1c}) and cognitive performance and their corresponding interaction term.

Metabolites	Concentration (i.u.)		Model 1						Model 2				Model 3			
			Type 2 diabetes status		Cognition status		Interaction		Fasting blood glucose		Interaction		HbA_{1c}		Interaction	
	Diabetes	Nondiabetes	β	p	β	p	β	p	β	p	β	p	β	p	β	p
GABA+[a]	1.68 ± 0.45	1.66 ± 0.39	0.626	0.041	0.057	0.800	-0.661	0.159	0.338	0.016	-0.381	0.104	0.316	0.026	0.464	<0.05
Glu	11.39 ± 1.15	11.31 ± 1.06	0.311	0.323	-0.133	0.572	-0.077	0.874	-0.022	0.880	0.220	0.379	-0.013	0.928	0.141	0.575

Concentrations are mean ± SD. Standardized β (95% confidence interval (CI)) indicates increments/decrements of the metabolites with type 2 diabetes status and cognitive status or the interaction term. The interaction term represents participants with both type 2 diabetes and less cognitive performance. GABA+, γ-aminobutyric acid; Glu, glutamate. i.u., institutional units; p, p-value. n=80, [a]n=79.

Model 1: adjusted for (dichotomous) type 2 diabetes status, (dichotomous) cognition status, age, sex, education level, BMI, percentage GM/WM ratio in voxel, and interaction between diabetes and cognition status. Model 2: model 1 with fasting blood glucose levels instead of the dichotomous type 2 diabetes status. Model 3: model 1 with HbA_{1c} levels instead of the dichotomous type 2 diabetes status. Note: cognition status is only reported for model 1 because the results did not differ for model 2 and 3.

^{1}H-MRS

Linear regression (Table 6.3) revealed higher GABA+ levels in participants with type 2 diabetes (p=0.041), but no differences in Glu concentrations. Linear regression analyses that included the interaction term (diabetes status x cognition status) revealed no significant interaction.

Additional linear regression analyses replacing type 2 diabetes status by fasting blood glucose levels or HbA_{1c} showed similar results (Table 6.3): higher GABA+ levels in participants with higher fasting blood glucose levels and in participants with higher HbA_{1c} levels. Furthermore, linear regression analyses that included the interaction term (HbA_{1c} levels times cognition status) revealed higher GABA+ levels in participants with both higher HbA_{1c} levels and less cognitive performance.

Post-hoc analyses

Post-hoc analyses (data not shown) revealed lower tNAA levels in participants who scored less on cognitive performance (β=0.504, p=0.030). Other metabolite concentrations did not show a significant analyses with type 2 diabetes or cognitive status (p>0.267). In addition, higher tCho (β=0.518, p=0.013) levels were observed in participants with both higher HbA_{1c} levels and less cognitive performance (interaction analyses).

Discussion

The current study examined whether neurotransmitter levels are related to type 2 diabetes, cognitive performance, and the potential interaction between diabetes and cognitive performance. The main findings were that GABA+ is higher in diabetes, as well as in participants with higher fasting blood glucose levels and in participants with higher HbA_{1c} levels, that GABA+ concentrations were higher in participants with both higher HbA_{1c} levels and less cognitive performance. To our knowledge, this is the first study to investigate the role of neurotransmitters in type 2 diabetes and cognitive decrements.

Higher GABA+ levels were found in participants with type 2 diabetes, in participants with higher fasting blood glucose levels, and in participants with both higher HbA_{1c} levels and less cognitive performance. Only one small diabetes study on GABA ^{1}H-MRS is available that reports lower GABA levels in patients with diabetic neuropathy, although translation of this result to the current type 2 diabetes population is not straightforward.[14] Nevertheless, increased GABA levels have been found in a type 2 diabetic rat model.[23] GABAergic inhibition is involved in the control of many behaviors, such as anxiety, psychosis, aggression, depression, mood, and cognition.[24] One possible explanation of how GABA affects cognition is by means of its

inhibitory function on dopamine release in the mesocortical dopamine pathway, which partly projects to prefrontal cortex. It has previously been hypothesized that this pathway regulates cognition and executive functions.[24] Thus, GABA could downregulate the dopamine activity which might eventually lead to impaired cognitive performance. This issue cannot be resolved with the results of the current study, future studies are needed to confirm this hypothesis.

No significant changes in Glu levels were found for participants with type 2 diabetes, less cognitive performance, nor their interaction, whereas a previous ^{1}H-MRS study did report decreased subcortical Glu levels in type 2 diabetes.[13] In contrary to our participants, these patients were suffering from a major depression, which could explain the difference in Glu concentrations. In addition, an important methodological difference is that the voxel placement and field strength (1.5T) differs compared with our study. Rather than excluding a potential role for the glutamatergic neurotransmitter system in less cognitive performance and type 2 diabetes, it could be the case that our applied technique for detecting Glu is simply not sensitive enough. An alternative ^{1}H-MRS technique, making use of spectral editing, might yield better results, due to an enhanced sensitivity.[25]

The post-hoc analyses revealed significant results regarding tNAA and tCho, and although this study was not designed specifically to investigate these metabolites, the results are still interesting. For participants who scored less on cognitive performance, we observed lower tNAA levels. tNAA is a surrogate marker of normal functioning neurons and these results indicate that a decline in neuronal integrity is associated with cognitive deficits. Similar results have been found in patients with mild cognitive impairment, which might suggest that tNAA could be a predictor for cognitive deficits.[26] With respect to cognition and type 2 diabetes, one ^{1}H-MRS study observed no differences in NAA metabolite concentrations and concluded that cognitive decline cannot be explained by this metabolite.[27] Nevertheless, this study was performed at lower field strength (1.5 Tesla), which could make the quantification less sensitive to NAA differences.

Furthermore, our study also observed higher tCho levels in participants with both higher HbA_{1c} levels and less cognitive performance. Choline is involved in membrane turnover, which is a process of loss and replacement of cellular membrane, inflammatory processes, astrocytosis, and in the synthesis of the neurotransmitter acetylcholine and all these processes could affect cognitive performance.[24,28] Interestingly, Sahin et al.[29] found higher choline levels in participants with poor glycemic control. Similar higher choline levels have been found in patients with Alzheimer's disease characterized by poor recognition memory performance.[30] Therefore, altered membrane metabolism seem to underlie cognitive decrements both in Alzheimer's disease and type 2 diabetes, which may indicate a shared mechanism.

This study has several strengths: first, to our knowledge, it is the first study to investigate the neurotransmitter system in participants with type 2 diabetes in

relationship with cognitive functioning. Second, the participants are extensively characterized. Third, the detection and quantification of the neurometabolites was applied at higher field strength (3T) and using editing, which results in improved spectral resolution, compared with 1.5T in most other studies in type 2 diabetes.

A number of limitations also need to be addressed. First, the study had a cross-sectional design, so the results should be interpreted cautiously in terms of causality. Nevertheless, the first results are promising and open directions to future longitudinal studies assessing neurotransmitter metabolism in type 2 diabetes and cognitive decrements. Second, our voxel was placed in the occipital lobe, chosen for optimal spectral quality, rather than neuropsychological relevance.[4] However, the fact that we do find significant effects, despite of the occipital placement of the voxel, might indicate that metabolic effects are global. Future studies could apply spectroscopic imaging to explore multiple brain regions to assess whether spatial variations could be related to specific cognitive domains.

Clinical perspectives

Unfortunately, our study was not able to measure cerebral insulin levels and therefore we were not able to link whether insulin causes directly the increase in GABA+ levels. Other studies already have shown the involvement of insulin on increased activity of dopamine neurons, and increased expression of the GABA receptors.[8,9,31] Thus insulin could also indirectly (independent of GABA+) be involved in the process of cognitive decrements in participants with type 2 diabetes. Therefore an important question to be addressed in future studies is whether insulin modulates directly the levels of GABA+ in the brain of participants with type 2 diabetes and causes cognitive decrements. Furthermore, our results open directions for future (longitudinal and pharmacological) studies, which are needed to unravel the underlying mechanism of cognitive decrements in type 2 diabetes. It would be interesting to investigate and validate whether induced changes in the GABAergic system or choline mechanism, for instance with drug therapies (GABAergic drugs or choline agonists), can lead to improvements in cognitive performance or prevention of cognitive deficits in participants with type 2 diabetes. Interestingly, drug treatment with Xanomeline, a muscarinic acetylcholine receptor agonist, has already been shown to provide promising results in improving cognitive performance in Alzheimer's disease, which was reflected by the normalization of cerebral choline to normal levels.[32]

In conclusion, this study revealed alterations in GABAergic neurotransmitter system in participants with type 2 diabetes, which were related to lower cognitive functioning.

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Chapter 7

Functional brain networks are altered in type 2 diabetes and pre-diabetes: Signs for compensation of cognitive decrements? - The Maastricht Study -

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Abstract

Type 2 diabetes is associated with cognitive decrements, accelerated cognitive decline, and increased risk for dementia. Participants with the metabolic syndrome, a major risk factor for diabetes, may display comparable cognitive decrements as seen in type 2 diabetes. Currently, the impact of (pre-)diabetes on cognition and the underlying organization of functional brain networks still remain to be elucidated. This study was designed to investigate whether functional brain networks are affected in type 2 diabetes and pre-diabetes. Forty-seven participants with diabetes, 47 pre-diabetic participants, and 45 matched control participants underwent detailed cognitive testing and 3-Tesla resting state functional MRI. Graph theoretical network analysis was performed to investigate alterations in functional cerebral networks. Participants with diabetes displayed altered network measures, characterized by a higher normalized cluster coefficient and a higher local efficiency compared with controls. The network measures of the pre-diabetic participants fell between those of the diabetes and control participants. Lower processing speed was associated with shorter path length and higher global efficiency. To conclude, participants with type 2 diabetes have altered functional brain networks. This alteration is already apparent in the pre-diabetic stage to a somewhat lower level, hinting at functional reorganization of the cerebral networks as compensatory mechanism for cognitive decrements.

Introduction

The worldwide prevalence of diabetes is increasing rapidly and the majority of patients are diagnosed with type 2 diabetes.[1] Along cardiovascular risks factors, type 2 diabetes is also associated with cognitive decrements, accelerated cognitive decline, and an increased risk for developing dementia and Alzheimer's disease.[2,3] A broad range of cognitive domains are affected in type 2 diabetes and one of the most commonly affected cognitive domains is processing speed.[4,5] However, the underlying pathological mechanism is not yet clear.

The progression of normal glucose metabolism to type 2 diabetes is a gradual process in which insulin resistance plays a crucial role. Insulin resistance, before the clinical presentation of type 2 diabetes, is often accompanied by other metabolic and vascular abnormalities. The cluster of these cardiovascular risk factors is referred to as the metabolic syndrome and is considered a major risk factor for developing diabetes.[6] Participants with the metabolic syndrome have a high likelihood to develop diabetes and may display comparable cognitive decrements as seen in patients with type 2 diabetes.[5] Furthermore, the cardiovascular risk factors are also associated with an increased risk of late-life dementia.[7]

Previous studies on cognitive decrements in diabetes using functional MRI (fMRI) have focused on changes in activation patterns, functional connectivity, and signal fluctuations. These diabetes studies revealed associations between impaired cognition, altered activation, and decreased functional connectivity.[8-14] Although functional connectivity was measured by correlating signal time-series of different and a priori selected cerebral regions, aberrant connections outside these predefined regions remain undiscovered. Moreover, the broad range of affected cognitive domains in type 2 diabetes suggests more widespread, global network disturbances, rather than perturbations of merely localized processes or specific functional networks. Since several domains are affected in type 2 diabetes, the decreased cognitive performance in individuals with type 2 diabetes might be due to a reduced efficiency or effectiveness of various processing resources.[15] In this context, novel brain connectivity analyses, which examine the integrity of networks in the entire brain, are appropriate.

Disturbances in local and global network organization (or efficiency) can be assessed by graph theoretical analysis.[16-18] In general, the brain can be thought as a graph, which is a collection of nodes (i.e. regions) and edges (i.e. functional connections between two regions). The organization of this graph can be characterized in terms of various so-called graph measures, including the cluster coefficient, local efficiency, characteristic path length, and global efficiency (Table 7.1). The cluster coefficient is a measure of local network connectivity and quantifies to which extent neighboring brain regions are also connected. A network with a high cluster coefficient contains densely connected local clusters. A measure which is closely related to the cluster coefficient is the local efficiency, which reflects the average efficiency of local clusters. The

characteristic path length is a measure of global network connectivity and represents the shortest average number of connections between any two brain regions. A network is considered highly efficient if the path length is relatively short and this is reflected in the graph measure global efficiency. It has been shown that the brain network organization typically behaves like a small-world network[18] and that this organization is disrupted in Alzheimer's disease.[19] Small-world networks are characterized by high cluster coefficient and a low characteristic path length[16] and are more efficient in synchronizing neural activity between different brain regions.[20]

How cognitive decrements in diabetes reflect alterations in functional brain organization needs to be resolved. In particular, it is unknown to what extent diabetes and pre-diabetes (i.e. metabolic syndrome with impaired glucose tolerance) share the same alterations in functional brain networks, or whether pre-diabetic alterations exist at all. Therefore, the current study was designed to investigate whether, and to which extent, global functional brain networks are affected in participants with type 2 diabetes and pre-diabetes.

Table 7.1 Description of the graph measures (normalized to random networks).

Graph measure	Symbol	Description
Cluster coefficient	γ	Quantifies the number of connections that exist between the nearest neighbors of a region as a proportion of the maximum number of possible connections.
Characteristic path length	λ	The minimum number of connections that must be traversed to go from one region to another.
Global efficiency	E_{global}	The average inverse shortest path length, which is inversely related to the characteristic path length.
Local efficiency	E_{local}	Mean of the global efficiency of subgraphs computed on the immediate neighbors of a region, which is related to the cluster coefficient.

Research design and methods

Study population and design

In this study, we used data from The Maastricht Study, an ongoing observational prospective population-based cohort study. The rationale and methodology have been described previously.[21] In brief, the study focuses on the etiology, pathophysiology, complications and comorbidities of type 2 diabetes mellitus and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known type 2 diabetes status for reasons of efficiency. The present report is derived from cross-sectional data from the first 3451 participants who

completed the baseline survey between November 2010 and September 2013. The baseline survey examinations of each participant were performed within a time window of three months. MRI was performed in a period of approximately 12.7±3.7 months later. The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Netherlands Health Council under the Dutch "Law for Population Studies" (Permit 131088-105234-PG). All participants gave written informed consent.

Of the 3451 participants, MRI measurements were available in 1109 participants (MRI assessments started from December 2013 onwards and is ongoing). From this group, we selected all participants with both the metabolic syndrome and impaired glucose tolerance (which was chosen to represent pre-diabetes) and matched them according to age, sex, and education level to participants with type 2 diabetes and participants with neither type 2 diabetes nor the metabolic syndrome and/or pre-diabetes. This resulted in 47 (pre-diabetic) participants with both the metabolic syndrome and impaired glucose tolerance, 47 participants with type 2 diabetes, and 47 participants without type 2 diabetes or the metabolic syndrome for this study. No incidental findings at radiological examination were reported in the selected participants.

Glucose metabolism status

All participants (except those who used insulin) underwent a standardized oral glucose tolerance test (OGTT). Participants were considered to have diabetes according to the WHO 2006 criteria if they use diabetes medication or if they had a fasting blood glucose ≥7.0 mmol/l or a 2-h blood glucose ≥11.1 mmol/l. Participants were considered to have both an impaired glucose tolerance and the metabolic syndrome if they had a fasting blood glucose <7.0 mmol/l and a 2-h blood glucose ≥7.8 and <11.1 mmol/l and if they did not use any diabetes medication or did not have type 2 diabetes and if they met two or more of the following criteria according to the adapted ATP III criteria[22]: waist circumference ≥88 cm for women and ≥102 cm for men; triglycerides ≥1.7 mmol/l; HDL cholesterol <1.3 mmol/l for women and <1.03 mmol/l for men; and blood pressure ≥130/85 mmHg or anti-hypertensive medication use. Participants without type 2 diabetes or pre-diabetes were defined by fasting blood glucose <6.1 mmol/l and a 2-h blood glucose <7.8 mmol/l and no use of diabetes medication.

Magnetic resonance imaging

For each participant, MRI data were acquired on a 3T clinical MR-scanner (MAGNETOM Prisma fit, Siemens Healthcare, Erlangen, Germany) using a 64-element head/neck coil for parallel imaging with an acceleration factor of two. The MRI protocol consisted of structural scans for radiological evaluation (including T1-weighted, T2-weighted, and fluid attenuated inversion recovery (FLAIR) sequence) and resting state fMRI scans.

Resting state fMRI data were acquired using echo-planar imaging pulse sequence sensitive to blood oxygen level-dependent (BOLD) contrast (TR/TE 2000/29 ms, flip angle of 90°, pixel size of 2x2 mm, slice thickness of 4 mm) and 195 volumes per acquisition. For anatomic references, a T1-weighted (T1) three-dimensional magnetization prepared rapid acquisition gradient echo (MPRAGE) (TR/TE 2300/2.98 ms, 1.00 mm isotropic voxel size, 176 continuous slices, matrix size of 256 x 240, field of view of 256 mm, inversion time of 900 ms) was acquired.

Data analysis

Image preprocessing

The T1 images were automatically segmented to obtain (sub)cortical gray and white matter areas, and cerebrospinal fluid (CSF) using the FreeSurfer software package (Martinos Center for Biomedical Imaging, Boston, USA)[23] and the segmentations were visually inspected. Data from two control participants were excluded due to image artifacts preventing reliable FreeSurfer segmentation and thus data from 47 participants with type 2 diabetes, 47 pre-diabetic participants, and 45 control participants remained suitable for the final analysis.

Image preprocessing of the time-series of the fMRI data was performed in the Statistical Parametric Mapping (SPM8) software package (The Wellcome Trust Centre for NeuroImaging, Neurology, University College London, UK). fMRI images were corrected for slice-timing and realigned to the mean image to correct for head movement. Then, these images were transformed to T1 space and spatially smoothed using a 4 mm full-width-half-maximum Gaussian kernel. Next, the individual anatomical T1 segmentations were registered to the fMRI images[24] to calculate averaged time-series for the (sub)cortical gray and white matter areas, and the CSF. To reduce the effect of physiological noise (respiratory and cardiac artifacts), the fMRI data was filtered using a band pass filter (0.01-0.1 Hz) and using linear regression with the averaged time-series of the white matter, CSF, and the movement parameters, acquired in the previous realignment step, as nuisance regressors.[25,26] Finally, the averaged time-series of the cortical and subcortical segmented areas[27,28] (a total of 82 areas, 41 for each hemisphere) were used to form a correlation matrix by calculating the Pearson correlation coefficients between all pairs of the segmented brain areas. Negative values were set to zero.[29] The correlation matrix stands for a brain graph in which each brain area represents a node, and each connection between brain area *i* and *j* represents an edge. For each participant, the brain graph was thresholded by a sparsity value (i.e. which percentage of the connections with the highest correlation coefficient were included while removing all other connections) based on the mean correlation matrix of the controls. In addition, for a given sparsity value the network (thus also the number of edges) was the same for each participant. Since no criteria are available for which sparsity value is the most biologically meaningful, a sparsity range of

0.1-0.9 with a step size of 0.01 was chosen, corresponding to 664-5978 connections. A high sparsity value represents a relatively low number of connections with the highest correlation coefficients.

Network parameters

For the global organization of large-scale networks, graph theoretical measures[16-18] were calculated from the brain graphs using the Brain Connectivity Toolbox[30] in Matlab (The Mathworks, Natick, Massachusetts, USA). The following graph theoretical measures were calculated (Table 7.1): *i)* the *cluster coefficient*, which is a measure of local network connectivity (i.e. a network with a high cluster coefficient is characterized by densely connected local clusters); *ii)* the *characteristic path length*, a measure of the average step length between two nodes (i.e. a network with low path length is characterized by short distances between two nodes); *iii) global efficiency*, a measure of the average inverse shortest path length and is inversely related to the path length; and *iv) local efficiency*, a measure of the mean of the global efficiency of subgraphs computed on the immediate neighbors of a node and it is related to the cluster coefficient. Furthermore, for each participant, the graph measures were normalized to comparable values from randomly generated networks (N=100)[16,17] to evaluated whether the network has small-world properties. Small-world networks are characterized by a high normalized cluster coefficient >1 and low normalized characteristic path length ≈1.

Covariates

Educational level was assessed by interview and classified into eight levels commonly used in the Netherlands: 1) no education; 2) primary education; 3) lower vocational education; 4) intermediate general secondary education; 5) intermediate vocational education; 6) higher general secondary education; 7) higher vocational education; and 8) university degree. For this study, educational level was subdivided into three groups: low (level 1 to 3), middle (level 4 to 6), and high (level 7 to 8). Office blood pressure was assessed three times on the right arm after a 10-minute rest, using a noninvasive blood pressure monitor (Omron 705IT, Omron, Japan). A fourth measurement was performed when the difference between measurement two and three exceeded more than 10 mmHg. Here, we used the averaged blood pressure values over all the available measurements.[21]

Cognition

Information processing speed was derived (*z*-score over the 3451 participants) from the Stroop Color Word Test (Part I and II)[31], Concept Shifting Test (Part A and B)[32] and the Letter-Digit Substitution Test[33] in which participants were instructed to match digits to letter as quickly as possible within 90 seconds. Global cognitive function was measured

by the Mini-Mental State Examination (MMSE) (score range 0-30).[34] Memory function was derived from the immediate and delayed recall Verbal Word Learning Test[35], in which 15 words are presented in five subsequent trials, followed by a recall phase immediately after each trial (score range 0-75), and a delayed recall phase after 20 minutes (score range 0-15). Executive functioning and attention was derived from Stroop Color Word Test (Part III)[31] and Concept Shifting Test (Part C).[32]

Statistical analysis

Descriptive participant characteristics are reported as mean ± standard deviation. Group characteristics were tested using one-way analysis of variance for continuous variables and pearson χ^2-tests for categorical variables. Post-hoc two-sided independent-samples *t*-tests and pearson χ^2-tests were calculated to test for significant group differences. Linear regression analyses were performed to assess the association of the graph measures with type 2 diabetes, pre-diabetes, and control participants (model 1). In addition, to evaluate whether the associations were robust, model 1 was extended with potential confounders (i.e. age, sex, education level, and systolic blood pressure; model 2). Finally, to assess whether the graph measures were associated with cognition, model 2 was further extended with information processing speed (model 3). Processing speed was chosen for this analysis, as it is mediated by multiple brain regions globally distributed throughout the entire brain.

Results

Table 7.2 shows the general characteristics of the participants. The groups were matched on age, sex, and education. As expected, the groups had different fasting blood glucose levels, HbA_{1c} levels, body mass index, waist circumference, systolic blood pressure, and cholesterol levels. Cognition scores were not significantly different. However, post-hoc analysis showed that participants with type 2 diabetes had significantly lower scores on information processing speed compared with the control participants.

Table 7.2 Clinical characteristics of participants with type 2 diabetes, pre-diabetes, and controls.

	Participants with type 2 diabetes (n = 47)	Participants with pre-diabetes (n = 47)	Control participants (n = 45)	p-value
Demographic factors				
Age (years)	61.0 ± 6.7	61.0 ± 6.7	60.7 ± 6.5	0.962
Sex, male (%)	51.1	51.1	51.1	1.000[a]
Education, low/middle/high (%)	46.8 / 25.5 / 27.7	46.8 / 21.3 / 31.9	44.4 / 24.4 / 31.1	0.983[a]
Alcohol consumption, none/low/high (%)	31.9 / 44.7 / 23.4	25.5 / 57.4 / 17.0	8.9 / 51.1 / 40.0[†,§]	0.023[a]
Smoking status, never/former/current (%)	29.8 / 48.9 / 21.3	21.3 / 59.6 / 19.1	33.3 / 60.0 / 6.7	0.238[a]
Cardiovascular disease (%)	10.6	6.4	11.1	0.689[a]
Type 2 diabetes-related characteristics				
Duration of diabetes (years)	8.7 ± 6.3*	-	-	
Newly diagnosed diabetes (%)[¶]	21.3	-	-	
Fasting blood glucose (mmol/l)	8.0 ± 2.2	6.0 ± 0.5[‡]	5.2 ± 0.3[†,§]	<0.001
HbA_{1c} (%)	6.8 ± 1.1	5.7 ± 0.4[‡]	5.4 ± 0.4[†,§]	<0.001
HbA_{1c} (mmol/mol)	51.2 ± 11.7	38.6 ± 4.7[‡]	35.8 ± 4.2[†,§]	<0.001
Fasting blood insulin (pmol/l)	95.8 ± 64.8	106.0 ± 50.5	52.3 ± 23.5[†,§]	<0.001
Type 2 diabetes medication		-	-	
Oral medication, yes (%)	70.2	-	-	
Insulin medication, yes (%)	14.9	-	-	
Insulin and oral medication, yes (%)	12.8	-	-	
Clinical variables				
BMI (kg/m^2)	29.4 ± 5.2	29.8 ± 4.0	25.4 ± 3.0[†,§]	<0.001
Waist circumference (cm)	104.0 ± 15.0	102.5 ± 10.3	90.4 ± 8.9[†,§]	<0.001
SBP (mmHg)	136 ± 14	141 ± 17	132 ± 17[§]	0.046
DBP (mmHg)	77 ± 8	79 ± 11	76 ± 8	0.131
Total cholesterol (mmol/l)	4.4 ± 1.0	5.5 ± 1.3[‡]	5.4 ± 1.1[†]	<0.001
HDL cholesterol (mmol/l)	1.4 ± 0.4	1.4 ± 0.4	1.8 ± 0.5[†,§]	<0.001
LDL cholesterol (mmol/l)	2.3 ± 0.9	3.2 ± 1.0[‡]	3.2 ± 0.9[†]	<0.001
Triglycerides (mmol/l)	1.8 ± 1.2	1.9 ± 1.0	1.1 ± 0.5[†,§]	<0.001
Cognitive score				
MMSE total score	28.9 ± 1.2	29.0 ± 1.3	29.1 ± 1.2	0.794
Memory function	-0.23 ± 1.00	-0.24 ± 1.08	0.03 ± 0.93	0.351
Information processing speed	-0.17 ± 0.80	-0.005 ± 0.86	0.19 ± 0.77[†]	0.109
Executive functioning and attention	-0.13 ± 0.86	-0.12 ± 0.86	-0.02 ± 0.77	0.782

Data are mean ± SD. HbA_{1c}, glycated hemoglobin; BMI, body mass index, SBP, systolic blood pressure; DBP, diastolic blood pressure; MMSE, Mini-Mental State Examination. Memory function, information processing speed, and executive functioning and attention are presented as mean standardized *z*-scores ± SD. *n=22, based on only the diabetes participants excluding the newly diagnosed diabetes participants. One-Way ANOVA; [a]Pearson χ^2-test; Post-hoc *t*-tests and Pearson χ^2-tests were calculated to test for significant group differences: [†]indicates p<0.05 between type 2 diabetes compared with controls; [‡]indicates p<0.05 between type 2 diabetes compared with pre-diabetes; [§]indicates p<0.05 between pre-diabetes compared with controls. [¶]As newly revealed by the OGTT.

Figure 7.1 displays the graph measures over a large range of sparsity values for the three groups. The networks of the three groups all exhibited a topology in the small-world regime with cluster coefficient larger than 1 and characteristic path length close to 1 (Figure 7.1AB). It can be appreciated that participants with type 2 diabetes display generally higher values for cluster coefficient, global, and local efficiency over almost

the entire sparsity range compared with controls, and pre-diabetic participants are intermediate between those two groups. Moreover, in type 2 diabetes these values of cluster coefficient and local efficiency were significantly higher for sparsity values (0.77-0.88 and 0.90, 0.62-0.90, respectively) compared with controls. For participants with pre-diabetes, local efficiency was also significantly higher over a large range of sparsity values (0.73-0.90) compared with controls. For characteristic path length, the control group displayed higher values compared with the other two groups, which was significantly higher for a small range of sparsity values (0.83-0.86) compared with the pre-diabetic group. No significant differences in graph measures were found between participants with type 2 diabetes and pre-diabetes. Table 7.3 shows the results of the linear regression analysis, performed for the graph measures corresponding to a sparsity value of 0.8. Supplementary Figure S7.1 shows the relative (to the control participants) differences in graph measures for the type 2 diabetes and the pre-diabetic group. All the results maintain intact after adjusting for age, sex, education level, and systolic blood pressure (model 2) (Supplementary Table S7.1).

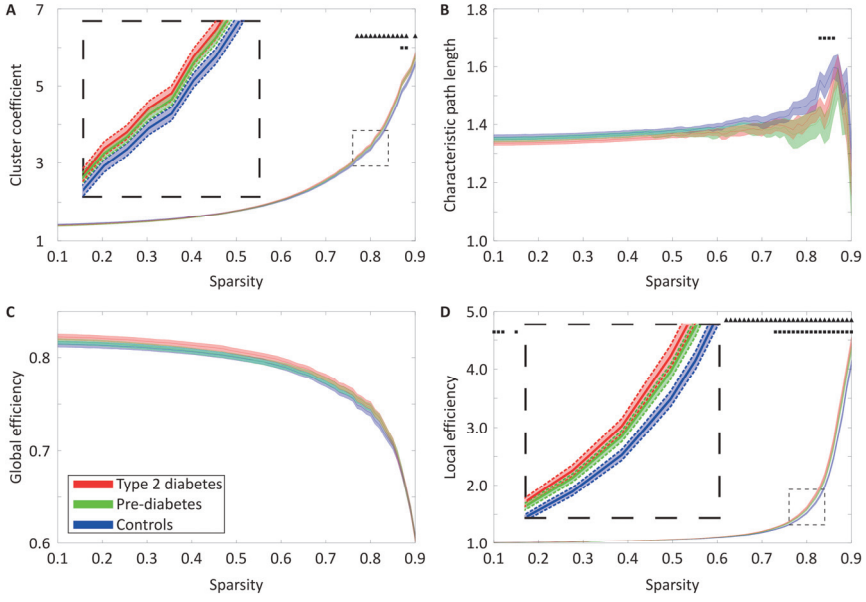

Figure 7.1 Network measures of the global organization of the entire cerebrum over a large range of sparsity values for participants with type 2 diabetes (red lines), pre-diabetes (green lines), and controls (blue lines). Straight lines indicate the mean values of the groups, dashed lines and the corresponding transparent areas represent the standard error of the mean. Black triangles indicate for which sparsity values participants with type 2 diabetes differ significantly from the controls. Black squares indicate for which sparsity values pre-diabetic participants differ from the controls. The normalized (to random matrices) graph measure are: **A**) cluster coefficient, **B**) characteristic path length, **C**) global efficiency, and **D**) local efficiency. All the results maintain significant after adjustment for potential confounders (model 2) (Supplementary Table S7.1).

Additional analyses incorporating information processing speed (model 3) revealed that increased characteristic path length was associated with improved processing speed, while an increased global efficiency was associated with lower processing speed (Supplementary Figure S7.2).

Table 7.3 Relationship of functional MRI graph measures in participants with type 2 diabetes and pre-diabetes, respectively, relative to healthy controls at a sparsity value of 0.8.

	Type 2 diabetes vs. controls			Pre-diabetes vs. controls		
Normalized graph measure	β	95% CI	p-value	β	95% CI	p-value
Cluster coefficient	0.106	0.007 to 0.204	0.036	0.074	-0.025 to 0.173	0.140
Characteristic path length	-0.054	-0.156 to 0.047	0.290	-0.079	-0.181 to 0.022	0.125
Global efficiency	0.008	-0.003 to 0.019	0.159	0.006	-0.005 to 0.017	0.285
Local efficiency	0.090	0.035 to 0.145	0.001	0.059	0.005 to 0.114	0.034

Unstandardized β (95% confidence interval (CI)) indicates increments / decrements of the graph measures with participants with type 2 diabetes or pre-diabetes. The model is not adjusted for potential confounders. Supplementary Table S7.1 presents the results of the model adjusted for potential confounders.

Discussion

This cross-sectional study is the first to evaluate global functional brain networks by exploring various graph measures in participants with type 2 diabetes, pre-diabetic participants, and matched control participants in one study. Graph theoretical network measures were compared between these groups, and in addition, the relation with processing speed was investigated. First, participants with type 2 diabetes displayed altered network parameters, including a higher normalized cluster coefficient and a higher local efficiency compared with controls. In addition, pre-diabetic participants also displayed higher normalized local efficiency compared with controls, though these values were qualitatively lower than in type 2 diabetes. Furthermore, no significant differences in network parameters were observed between pre-diabetic participants and type 2 diabetes, and all the groups exhibited small-world organization of their networks indicative of an efficient cerebral topology. Cognitive performance was similar for the three groups, and lower information processing speed was associated with a lower normalized characteristic path length and with a higher global efficiency.

In normal brain networks, the combination of a high cluster coefficient and a high local efficiency reflects a high local specialization of the brain in processing information. We observed this combination in participants with type 2 diabetes, suggesting that the brain is better organized as compared with the cerebral networks of the controls. In view of the known effect of diabetes on cognition, such as cognitive decrements and higher risk to develop dementia or Alzheimer's disease, these results may seem contradictory, as one could expect that the cerebral networks are less, rather than better, organized in diabetes. It is important to note that the included diabetes population in this study is relatively healthy in terms of their mildly affected cognitive

performance (in the range of normal performance), and in terms of good treatment control with regard to glucose levels. A better organized cerebral network in diabetes can be attributed to an earlier stage of structural brain damage, where compensatory mechanisms, such as functional reorganization of networks, might play a role.[36] In addition, all the four network measures investigated in this study showed the same direction, thus supporting an underlying compensatory mechanism. To further support the compensatory interpretation, additional analysis observed no effect of age in our study population (data not shown), which is known to have an impact on the network parameters.[37,38] Interestingly, increased functional connectivity was previously observed in type 1 diabetes patients without retinopathy compared with healthy controls.[39] In addition, graph theoretical analysis on diffusion data already showed disrupted white matter networks in type 2 diabetes[40], and it will be interesting to investigate the relationship with our findings.

Our findings with respect to the pre-diabetic participants, who showed intermediate topological values of the functional networks, also strengthens the explanation of an underlying compensatory mechanism. Therefore, a higher local efficiency might be an early marker for cognitive decrements that may eventually lead to brain degeneration. It was previously shown that patients with Alzheimer's disease showed a decline of the small-world network organization, characterized by decreased normalized cluster coefficient, and a decrease in local efficiency, indicative of disrupted local network connectivity.[19] Taken together, a hypothetical overview over time (Figure 7.2) illustrates that before the onset of clinically manifest cognitive decrements, functional reorganization of the cerebral networks may already have started as a compensatory mechanism to counteract the slight decrements in cognitive performance in participants with pre-diabetes and type 2 diabetes. Then, when the functional reorganization fails, functional networks will get disrupted and the first signs of cognitive decrements will be recognizable in participants with diabetes. Finally, this might possibly explain the increased risk to develop in a later stage mild cognitive impairment and Alzheimer's disease. It remains to be explored how the graph theoretical measures behave in diabetes participants with more severe cognitive decrements or mild cognitive impairment[3] (Figure 7.2, gray area), as those results could support the presumed underlying compensatory mechanism.

In normal brain networks, the combination of a shorter characteristic path length and a higher global efficiency reflects the great ability of the brain to globally integrate information. We observed that this combination was associated with less processing speed and that participants with type 2 diabetes scored lower on processing speed compared with controls (Table 7.2). These results also fully complement the hypothetical underlying compensatory mechanism (Figure 7.2).

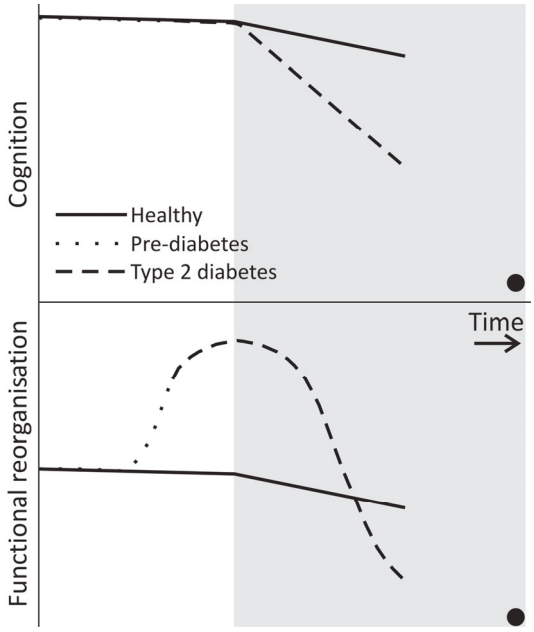

Figure 7.2 Hypothetical overview of cognitive performance and functional reorganization over time. Before the onset of recognizable cognitive decrements, functional reorganization of the cerebral networks already has started as a compensatory mechanism for the (slight) decrements in cognitive performance in participants with pre-diabetes (dotted line) and type 2 diabetes (dashed line). When the functional reorganization fails, the functional networks will get disrupted and the first signs of cognitive decrements will manifest. Finally, this might possibly explain in a later stadium the increased risk to develop dementia. The white area indicates the area of our study findings in control participants, pre-diabetes, and type 2 diabetes, while the gray area is still an unexplored open study area for which longitudinal studies can be beneficial. The black dots represent previously reported findings in patients with Alzheimer's disease.[19] Notably, not everyone with pre-diabetes develops diabetes and not everyone with diabetes develops Alzheimer's disease.

Supplementary Figure S7.3 plots the local efficiency as a function of fasting blood glucose level. It can be seen that the local efficiency increases with blood glucose level. As our results indicate, the effect of a high blood glucose level on local efficiency is already apparent in the pre-diabetic stage. As it has previously been shown that improvement in glycemic control in type 2 diabetes is associated with improved cognition[41], a good management of the blood glucose, also in the pre-diabetic stage, seems essential to reduce its impact on the brain. This way, the onset of cognitive decrements may be postponed or, hopefully, halted. It is important to note that we should not neglect the (additional) role of the other metabolic syndrome factors on the local efficiency. A post-hoc analysis revealed that participants with type 2 diabetes who

also meet the criteria for the metabolic syndrome displayed even higher local efficiency values (data not shown).

It has been shown that the BOLD signal is likely affected in type 2 diabetes due to an impaired vascular reactivity as a consequence of an altered hemodynamic response and changes in neurovascular coupling.[42] It remains to be elucidated how exactly changes in the hemodynamic response translate to changes in functional connectivity and network efficiency.

This study has several strengths: first, to our knowledge, it is the first study to investigate graph theoretical network measures in participants with pre-diabetes as well as type 2 diabetes, compared with matched control participants. Second, the participants have been extensively characterized. As correction for age, sex, and blood pressure did not change the significant results, we are confident that these factors do not underlie the observed differences between groups.

Our study has also some limitations: first, the study has a cross-sectional design, therefore the results should be interpreted cautiously in terms of causality. Nevertheless, the first results provide new insights and also prompt new questions for future studies to investigate the behavior of network measures over time. Second, due to the inclusion of a relatively healthy study population we were not able to investigate the full range of the hypothetical curve, as diabetic participants with severe diabetes complications did not participate in the study. Therefore, it will be interesting to investigate the functional network properties of diabetic participants with severe complications.

To conclude, this study reveals altered functional brain networks in type 2 diabetes as well as pre-diabetic participants, indicative of a more efficient cerebral organization. This was observed in diabetic and pre-diabetic participants with mild cognitive decrements, which are still in the range of normal cognitive functioning. Notably, the functional network measures of the pre-diabetic group are intermediate between participants with type 2 diabetes and controls, hinting at compensation for cognitive decrements in terms of functional reorganization of the cerebral networks. Furthermore, the study shows that functional graph network measures provide new insights and have the potential to serve as an early MRI biomarker for subtle cerebral alterations in relation to cognitive decline.

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Supplementary data

Table S7.1 Relationship of functional MRI graph measures in participants with type 2 diabetes and pre-diabetes, respectively, relative to healthy controls at a sparsity value of 0.8.

	Cluster coefficient		Characteristic path length		Global efficiency		Local efficiency	
	β	p-value	β	p-value	β	p-value	β	p-value
Type 2 diabetes	0.115	0.023	-0.053	0.312	0.007	0.193	0.095	<0.001
Pre-diabetes	0.093	0.071	-0.075	0.157	0.005	0.379	0.070	0.015
Age	0.004	0.272	0.004	0.241	6.2e-05	0.860	0.003	0.117
Sex (female = 1)	0.019	0.653	0.045	0.298	-0.008	0.068	-0.002	0.949
Education	0.024	0.324	0.011	0.669	-0.003	0.267	-0.005	0.700
Systolic BP	-0.002	0.085	-6.5e-05	0.646	1.1e-04	0.452	-0.001	0.063

Unstandardized β indicates increments/decrements of the graph measures with participants with type 2 diabetes, pre-diabetes, and potential confounders (model 2).

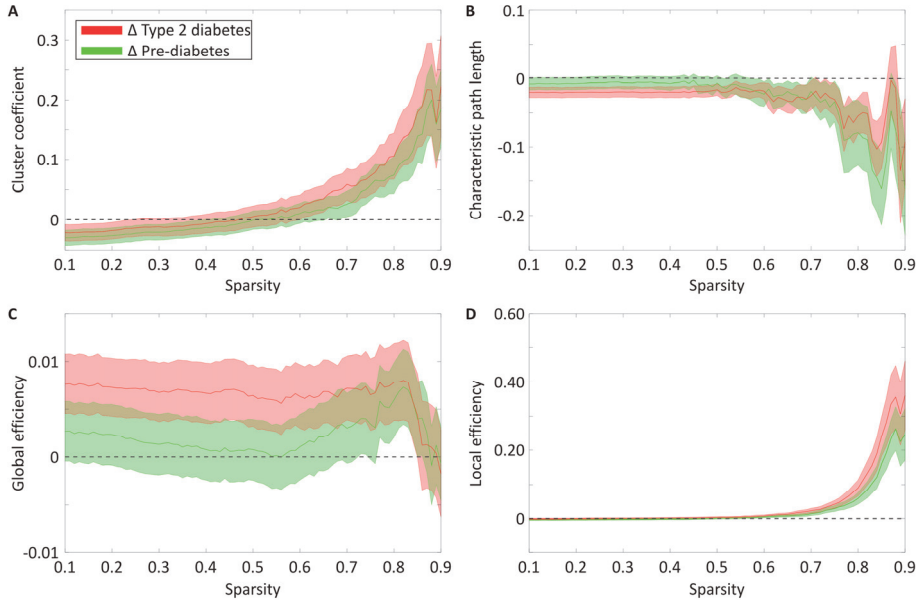

Figure S7.1 Relative network measures of the global organization of the entire brain over a large range of sparsity values for participants with type 2 diabetes (red lines) and pre-diabetes (green lines), both relative to the control participants. Straight lines indicate the relative (to the controls) mean values of the groups, colored dashed lines and the corresponding transparent areas represent the standard error of the mean. Dashed black lines indicate the zero line. **A**) normalized cluster coefficient, **B**) normalized characteristic path length, **C**) normalized global efficiency, and **D**) normalized local efficiency.

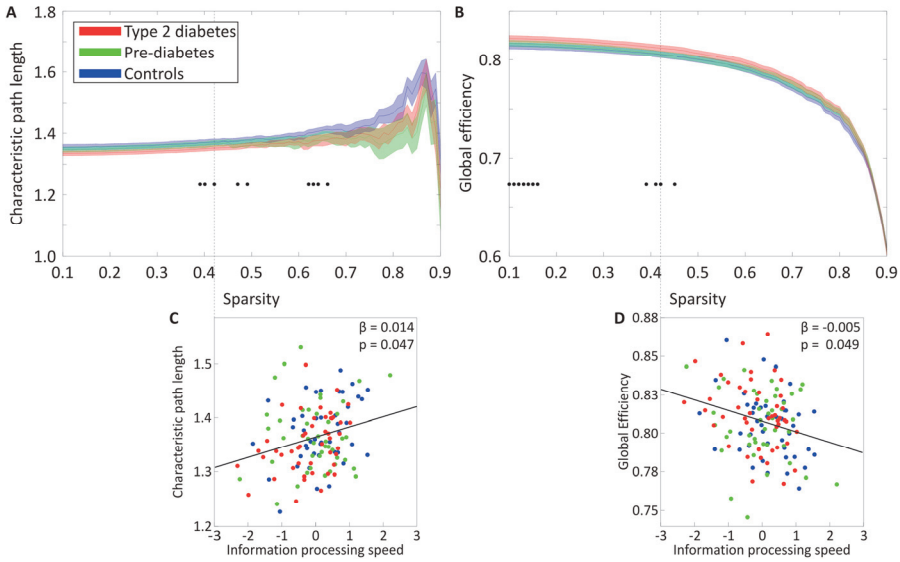

Figure S7.2 Network measures of the global organization of the entire brain over a large range of sparsity values which are associated with information processing speed after adjusting for potential confounders (model 3). Red lines (straight and dashed) indicates the mean and standard error of the mean for participants with type 2 diabetes. Green lines represents participants with pre-diabetes, blue lines represents the control participants. Black dotes indicate for which sparsity value processing speed was significantly associated with the graph measures. **A**) increased normalized characteristic path length was associated with improved processing speed, **B**) increased global efficiency was associated with less processing speed. Corresponding scatter plots (**C** and **D**) of the associations between graph measure and processing speed for graphs **A** and **B**, respectively, at a sparsity value of 0.42 are shown, with the regression line in black. Red, green, and blue dots in both scatter plots represent participants with diabetes, participants with pre-diabetes, and control particpants, respectively. Notably, information processing speed values are presented as mean standardized *z*-scores.

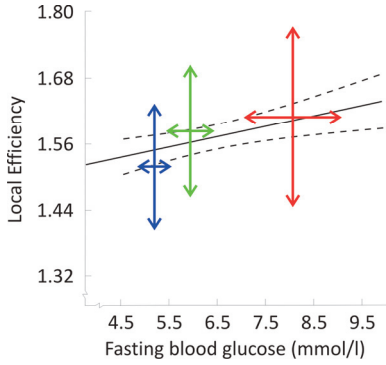

Figure S7.3 The relationship between local efficiency by a sparsity value of 0.8 and fasting blood glucose levels in participants with type 2 diabetes (red lines), pre-diabetes (green lines), and controls (blue lines). The intersection of the lines represent the mean values and the arrows represent the standard deviations. The efficiency increases with fasting blood glucose level.

Chapter 8

General discussion

Type 2 diabetes is associated with cognitive decrements, accelerated cognitive decline, and an increased risk for developing dementia and Alzheimer's disease. The exact neuronal mechanisms underlying cognitive decrements in diabetes still remain to be elucidated. The main aim of the research described in this thesis was to find neuronal MRI correlates of cognitive decrements in type 2 diabetes by applying advanced MR neuroimaging techniques.

This chapter summarizes, combines, and discusses the main findings of the previous chapters on advanced MRI examinations in diabetes. Subsequently, the clinical implications and some methodological considerations are addressed. Finally, directions for future research will be given.

Main findings

The review in chapter 2 provides an overview of cerebral abnormalities in type 2 diabetes and the neuroimaging techniques to study the pathophysiology ranging from routine clinical application to explorative research. In this review it is concluded that routine clinical MRI has extensively been applied to study structural abnormalities, whereas more-advanced MRI techniques were used too scarcely yet. In this thesis, we, therefore, applied a versatile set of advanced MRI techniques, expected to provide additional insights with respect to routine clinical MRI.

Novel cerebral abnormalities in type 2 diabetes

One of the possible underlying causes that may explain the link between type 2 diabetes and cognitive decrements, in particular related to memory function, was investigated using diffusion MRI (dMRI) in chapter 3. Using dMRI, abnormalities in the local hippocampal microstructure and abnormalities between the white matter connectivity (i.e. tract volume) of the hippocampus to other brain regions were assessed. Tractography revealed reduced white matter connectivity between the hippocampus and frontal lobe in participants with type 2 diabetes. No differences in the local microstructure were observed. Chapter 4 also examined the hippocampus, but here, in addition to the local microstructure, microvascular properties were investigated with intravoxel incoherent motion (IVIM) MRI, another diffusion-based technique. Especially the microvascular properties were found to be altered in type 2 diabetes, in terms of an increased blood perfusion volume and increased blood flow. Whole brain perfusion and the (bulk) blood supply from the common carotid artery to the brain were investigated in chapter 5. Hypoperfusion, measured with arterial spin labeling (ASL) MRI, was observed in type 2 diabetes, particularly in the subcortical gray matter, while no diabetes specific effects were observed for the carotid blood supply to the brain or cerebral blood flow in any other cortical brain region. In chapter 6 it was

investigated whether abnormalities in the inhibitory and excitatory neurotransmitters, i.e. γ-aminobutyric acid (GABA) and glutamate, respectively, were related to diabetes. While glutamate levels did not show an effect, the GABA levels were higher in participants with type 2 diabetes. Chapter 7 investigated participants with diabetes, pre-diabetes, and matched controls and in these groups abnormalities in functional networks were determined using graph theoretical network analysis on resting state functional MRI (fMRI) data. Participants with type 2 diabetes showed a more efficient cerebral organization compared with matched controls and the pre-diabetic participants showed intermediate results between those two groups.

Cognition and MRI abnormalities

The associations between the outcomes of the various neuroimaging techniques with cognitive performance were also examined. In chapter 3, we observed fewer hippocampal white matter connections to the temporal lobe in participants who scored relatively low on memory function. Negative associations were reported between memory performance and hippocampal microstructure (i.e. higher diffusion coefficient for reduced memory performance) and microvascular blood flow in chapter 4, while no associations were found between cognition and cerebral or carotid blood flow (chapter 5). MR spectroscopy (MRS) (chapter 6) revealed lower *n*-acetyl aspartate levels in participants who scored less on cognitive performance. Furthermore, in chapter 7 it was shown that a more efficient cerebral organization (i.e. shorter path length and higher global efficiency) was associated with lower processing speed.

Diabetes, MRI abnormalities, and cognition

The combined effect of diabetes and cognition was examined using an interaction analysis to provide additional insight into the underlying mechanisms more specific for diabetes. Participants with both diabetes and lower cognitive function showed alterations in the hippocampal microvasculature (i.e. higher microvascular pseudodiffusion and blood flow) (chapter 4) and cell metabolism (i.e. higher GABA and choline levels) (chapter 6).

Potential (early) neuronal MRI correlates

In our opinion, the outcomes of the IVIM, MRS, and fMRI techniques have the potential to serve as early neuronal MRI correlates of cognitive decrements in type 2 diabetes. These techniques already showed abnormalities in participants with type 2 diabetes who scored less on cognitive performance, while the studied population was relatively healthy in terms of their mildly affected cognitive performance. Please note that the cognitive scores of the studied participants were still in the range of normal performance.[1]

In more detail, IVIM revealed higher hippocampal microvascular pseudodiffusion and blood flow in participants with both higher fasting blood glucose levels and lower memory performance, which could be indicative for alterations of the microvasculature or an increased permeability of the vessel wall. The latter explanation is in agreement with a previously reported study on increased blood-brain barrier permeability in patients with type 2 diabetes.[2]

MRS showed higher GABA and choline levels in participants with type 2 diabetes who scored lower on cognitive tests. GABA is an inhibitory neurotransmitter and is involved in cognitive processes, but also in the control of many behaviors like aggression, anxiety, depression, mood, and psychosis.[3] Choline could affect cognitive performance as well due to its involvement in membrane turnover, astrocytosis, synthesis of the neurotransmitter acetylcholine, and inflammatory processes.[3,4] In addition, higher choline levels were already found in participants with poor glycemic control and patients with Alzheimer's disease,[5] which may indicate a shared mechanism between diabetes and Alzheimer's disease, and therefore choline has the potential to be an early biomarker.

fMRI showed that functional network based approaches reveal a more efficient cerebral organization in type 2 diabetes compared with controls, and participants with pre-diabetes were intermediate between those two groups. These early alterations in functional networks of participants with (pre-)diabetes hint at an early sign of cerebral adaptation, for instance, compensation.

Neuronal mechanisms

Vascular mechanism

Although the various MRI techniques that were described in this thesis focus on different aspects of brain tissue, some of these techniques have a certain overlap in terms of the investigated underlying mechanism. For example, IVIM and ASL investigated both the role of an underlying vascular mechanism. In addition, fMRI is also based on a vascular mechanism as brain activity is measured through changes in blood oxygenation (neurovascular coupling). As mentioned in the introduction, diabetes is associated with both macrovascular and microvascular complications[6-8], which already suggests the involvement of a vascular mechanism. Altered cerebral hemodynamics was thought as one of the potential mechanisms underlying cognitive decrements in diabetes.[9] Therefore it was interesting to investigate whether we were able to identify some early (subtle) cerebral alterations, which could be linked to this vascular mechanism. The outcomes of the IVIM and fMRI techniques suggest a compensatory act to the subtle cerebral alterations based on an underlying vascular mechanism. The first technique revealed increased hippocampal blood flow in diabetes,

which could be indicative of alterations (i.e. vasodilatation or more tortuous vessels) or an increased permeability of the microvasculature. Our hypothesis of a compensatory mechanism was based on other studies that observed a similarly high hippocampal blood flow in patients with mild cognitive impairment[10,11], but low hippocampal blood flow in patients with Alzheimer's disease.[10]

The results of the fMRI, based on the underlying neurovascular coupling, showed that the brain in participants with diabetes and pre-diabetes was more efficiently organized than controls which can be attributed to an early stage of brain adaptation. In this context, functional reorganization of the networks may play a role before the onset of recognizable cognitive decrements.[12] To strengthen the idea of compensation, a previous study showed a less efficient organized functional network in patients with Alzheimer's disease.[13]

Increased blood perfusion and blood flow was found with IVIM in the hippocampus, while ASL showed hypoperfusion in the subcortical gray matter but not the hippocampus. Explanations for these different results might be based on the conceptual differences in physiological contrast mechanisms and sensitivities of the two different MRI methods.[14] Therefore, the IVIM and ASL perfusion values cannot directly be compared to each other. On the one hand, to properly quantify the IVIM perfusion values, full knowledge of the intra-individual capillary geometry (i.e. the capillary segment length and the total capillary length) within a given voxel is required, which is impossible to measure noninvasively.[15] On the other hand, the IVIM signal is thought to come from intra-voxel properties and is, for instance, independent of the arterial arrival time of blood (i.e. the time between the magnetic labeling of the blood and its arrival in a certain region) which is used by ASL. As the labeled blood decays with the longitudinal relaxation time (T1) of blood, it is difficult to accurately quantify the ASL perfusion in regions with long arterial arrival times such as the white matter. In addition, no regions other than the hippocampus were investigated with IVIM in this thesis, which makes the comparison with the ASL subcortical gray matter region not possible. Nevertheless, both the IVIM and ASL results support the hypothesis of a vascular pathophysiologic mechanism in diabetes.

Glucose-mediated mechanism

The relation between fasting blood glucose and HbA_{1c} levels on the MRI outcomes was also investigated. Higher fasting blood glucose levels were associated with increased hippocampal blood perfusion and blood flow, higher GABA levels, and increased local cerebral efficiency, while a trend was observed with reduced hippocampal white matter connectivity to the frontal lobe. Higher HbA_{1c} levels were associated with higher GABA levels, and a trend of reduced white matter connectivity between the hippocampus and the frontal lobe. Above results indicate the involvement of an underlying glucose-mediated mechanism and it would be clinically relevant to evaluate how these MRI outcomes will behave after improving the blood glucose levels.

Other mechanisms

In this thesis we could not investigate all the other possible underlying mechanisms, for instance the direct involvement of insulin or inflammation such as measured with advanced glycation end-products (AGEs), soluble intercellular and vascular adhesion molecules (sICAM and sVCAM, respectively) and/or high-sensitivity C-reactive protein (hs-CRP) levels.[16-19] Although we observed higher GABA levels in diabetes and other studies have shown the involvement of insulin on the increased expression of the GABA receptors[20,21], the direct involvement of an underlying insulin mechanism still needs to be addressed in future studies.

Clinical implications

Based on the MRI findings in this thesis, ultimately one would like to provide an individual prognosis of patients with diabetes who are at high risk for cognitive decline. Unfortunately, this is not possible yet. This thesis provides insight into the (early) neuronal substrates of cognitive decrements and might open new opportunities to monitor therapeutic/lifestyle interventions for improving cognition and/or prevention of cognitive decrements.

As mentioned above, a vascular mechanism and a neurovascular coupling mechanism, which both possibly act compensatory, and a glucose-mediated mechanism seem to play an important role in the observed cognitive decrements. In addition, these mechanisms are assumed to be inextricably linked to each other. This suggests that treatment to prevent decline of or improve vascular function and/or blood glucose levels could be beneficial in patients with type 2 diabetes. The advanced MRI techniques have the potential to monitor, for instance, the effects of improving blood glucose levels on the brain. As promising results showed that cognitive performance improved by a better regulation of glycemic blood levels[22], it will be interesting to see whether this has also a positive effect on the MRI outcomes to further unravel the underlying mechanism of cognitive decrements.

Besides blood glucose levels, other criteria of the metabolic syndrome such as triglyceride levels, cholesterol levels, blood pressure, and waist circumferences are also important as participants with type 2 diabetes who also met the metabolic syndrome criteria displayed even greater alterations in the functional networks (chapter 7). Furthermore, individuals with the metabolic syndrome have been shown to be associated with lower cognitive performance[23] and the presence of multiple cardiovascular risk factors was associated with a higher risk to develop late-life dementia.[24] Interestingly, it has been shown that lifestyle interventions were effective in preventing or delaying (for up to 10 years) diabetes among older participants[25,26], but if or which lifestyle intervention is the most effective for the brain to prevent a decline of or improve cognitive function needs further research.

Next to the control of glucose levels or metabolic profile, another possibility, which might be beneficial, is to improve the vascular function with medication (i.e. antihypertensive or antiplatelet drugs) aimed at the vascular risk factors. In addition, other drug therapies (i.e. GABAergic drugs or choline agonists) can induce changes in the GABAergic or choline mechanisms, which might have a beneficial effect on improving cognitive performance or preventing further cognitive decrements. Promising results to improve cognitive performance have already been reported by treatment with the drug Xanomeline, which is a muscarinic acetylcholine receptor agonist, in patients with Alzheimer's disease by returning cerebral choline back to normal levels.[27] However, these treatment possibilities should first be extensively studied before they could be used in patients with type 2 diabetes.

Methodological considerations

The findings of the present thesis need to be discussed in light of some methodological considerations. First of all, the participants included in the studies were all recruited or selected from data of the ongoing Maastricht Study. As participants in The Maastricht Study underwent an extended battery of multiple elaborate measurements conducted over four/five different visits on separate days, selection bias could have been introduced because only participants who were able and willing to participate were included. This (may have) resulted in a relatively healthy, well-educated and young (mean age of 59.8 years) study population. In addition, participants with type 2 diabetes were also relatively healthy in terms of the mean diabetes duration (8.5 years) and, approximately, 25% had a known history of cardiovascular disease compared with other studies in which participants had approximately a mean diabetes duration of 10.4 years and 29-53% had prior cardiovascular disease.[28,29] It is therefore likely that the more severe patients with type 2 diabetes were not included in The Maastricht Study, which possibly could point out an underestimation of more general diabetes effects.

Second, the research described in this thesis is based on a cross-sectional design. Therefore, it is difficult to draw conclusions in terms of causality. Longitudinal studies are needed to make more firm conclusions regarding causality. They could help to investigate whether the suggested underlying compensatory mechanism for cognitive decrements of the form of a functional reorganization of the cerebral networks (Figure 7.2) is stable or varies over time and has the potential to serve as an early MRI biomarker.

Next, the results were based on two different study designs (Figure 1.2). In four chapters (Chapter 3-6) the selection of participants was based on cognition, while in chapter 7 the selection was based on (pre-)diabetes status. The study design based on cognition has both strengths and weaknesses. One point which could be considered

either as a strength or a weaknesses is the division of participants in a lower and higher cognitive performance group. It is known that cognitive decrements are subtle and develop slowly over time in type 2 diabetes[30], and that a clinically manifest effect of diabetes on cognition mainly occurs in patients who are older than 65 years.[31] For these reasons and as a consequence of a relatively healthy study population in The Maastricht Study, our studies (chapter 3-6) focused on participants with the lowest and highest cognitive performance to increase the possibility of identifying neuronal substrates of subtle cognitive decrements in diabetes. However, the weakness of this design is that this selection has lead to differences in clinical characteristics between participants with and without diabetes (Table 4.1). Therefore, our study population was relatively heterogeneous with respect to diabetes status. Although it reflects typical participants with and without type 2 diabetes, correction for age and sex was necessary in the statistical analyses.

The study design with the selection of participants based on (pre-)diabetes status includes well-matched participants with diabetes, pre-diabetes, and participants neither with diabetes or pre-diabetes (Table 7.1). Nevertheless, this population did not have a large dispersion in the cognitive measures, which reduced the probability of identifying possible early neuronal substrates of cognitive decrements.

Another limitation is that the time span between the cognitive performance tests and the MRI assessments was 16.1 and 12.7 months for the selection of participants based on cognition (Chapter 3-6) and (pre-)diabetes status (Chapter 7), respectively. Preferably, these time spans should be as short as possible. However, it is not possible to achieve shorter time spans with an extensive population study as The Maastricht Study.

A strength of this thesis is that we applied versatile and advanced MRI techniques. These techniques focus on number of physiological aspects of the brain and therefore provide more insight into the different ongoing physiological processes and mechanisms of the disease. Nevertheless, another constraint is that the voxel for spectroscopy was placed in the occipital lobe. This region was chosen for optimal spectral quality, rather than neuropsychological relevance. In addition, the dMRI and IVIM results were restricted to only the hippocampus or connections seeded from the hippocampus, while ASL and fMRI had a whole brain approach. Nonetheless, whole brain approaches could easily be implemented in the future.

Directions for future research

The findings of the present thesis demonstrated that advanced MRI techniques were able to identify neuronal changes of cognitive decrements in type 2 diabetes. These promising findings were evaluated in a cross-sectional design, and ideally longitudinal studies should be performed to assess the temporal aspects of these findings. Validating these potential MRI biomarkers might open new avenues for intervention

studies. Ideally, to find or validate MRI biomarkers of cognitive decrements, future studies are needed that include both participants with pre-diabetes, (elderly) patients with more severe diabetes and patients with mild cognitive impairment or dementia. This will lead to more insights into the time course of the disease. As higher blood glucose levels are associated with an increased risk to develop dementia[32], it would be worth studying whether a better regulation of the glycemic blood levels has a beneficial effect on cognition and our MRI findings.

Future studies should keep in mind that diabetes is a (non-focal) systemic disease, rather than a focal disease such as (partial) epilepsy and stroke, and, therefore, some 'standard' analysis methods might be less appropriate, as shown in chapter 5. It was shown that the alternative novel analysis approach was able to detect hypoperfusion in type 2 diabetes, whereas the more 'standard' analysis methods appeared to be not sensitive enough. Thus, future studies should explore alternative, and possibly more adequate or sensitive, analysis methods to unravel the underlying mechanisms of cognitive decrements in diabetes.

The presented studies in this thesis started to unravel the underlying mechanisms of cognitive decrements, however, this process has only just begun. As mentioned above, placing the spectroscopic voxel in regions of cognitive relevance such as in the frontal or temporal lobes (i.e. the hippocampus) is recommended. The technological and methodological development in the MRI world is still ongoing and nowadays even whole brain multi-voxel spectroscopy is possible which would definitely increase the potential scientific and maybe, eventually, the clinical utility. Moreover, high field MRI (>3.0 Tesla) might also be considered to improve for instance the spectral resolution in MRS, although high field entails other problems such as field inhomogeneity artifacts.

Conclusion

This thesis shows the benefits of applying various advanced MRI techniques to explore the underlying mechanisms of cognitive decrements in type 2 diabetes. Even before the onset of clinically manifest cognitive problems or structural brain damage in type 2 diabetes, slight decreases in cognitive performance are reflected by subtle cerebral alterations, identified by these techniques. Both vascular-mediated and glucose-mediated underlying mechanisms, inextricably linked to each other, are involved in this pathophysiology. Thereby, the vascular and neurovascular coupling mechanisms might act compensatory to the subtle cerebral alterations, which ultimately will be insufficient and therefore worsen cognitive performance. The use of advanced MRI techniques, including microstructural, microvascular, metabolic, and functional MRI, is a valuable way to gain more insight into the underlying mechanisms, which hopefully leads to the development and evaluation of better treatment or intervention strategies to delay or even prevent the worsening cognitive decrements in patients with type 2 diabetes.

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Summary

Type 2 diabetes is a common metabolic disorder, characterized by chronic hyperglycemia, in a context of insulin resistance and relative insulin deficiency and has a broad range of clinical complications. One of these complications is that type 2 diabetes is associated with cognitive decrements, accelerated cognitive decline, and an increased risk to develop dementia and Alzheimer's disease. Today we have neither tests nor biomarkers available to show who will decline cognitively and who will not. In addition, the exact neuronal mechanisms underlying the cognitive decrements still remain to be elucidated. As conventional MRI techniques are able to detect macrostructural, relatively end-stage effects of impaired cerebral tissue, these MRI techniques are probably not sensitive enough to detect and unravel early cerebral changes associated with cognitive decrements. Novel, advanced MRI techniques provide the opportunity to detect abnormalities in more detail at the microstructural, microvascular, metabolic, and functional level. The research described in the present thesis was aimed at finding neuronal MRI correlates of cognitive decrements in type 2 diabetes by applying these advanced MR neuroimaging techniques.

Chapter 1 provides a general introduction on diabetes and its relation with cognition and MRI. Furthermore, it includes a description of the study designs applied in the present thesis.

In **chapter 2**, we present a narrative review of the literature on various (potentially) pathological brain abnormalities associated with type 2 diabetes and cognitive decrements. In addition, this review also illustrates the dedicated neuroimaging techniques to study the pathophysiology ranging from routine clinical (conventional MRI) application to explorative research (advanced MRI). It is concluded that multifactorial pathophysiological processes are involved in the underlying cognitive decrements and that conventional MRI has extensively been applied to study structural abnormalities, whereas advanced MRI techniques have currently been used too scarcely. The identification of biomarkers are needed to study the link between brain abnormalities, diabetes, and cognitive decrements, which can to some extent be provided by these advanced MR neuroimaging techniques.

In **chapter 3**, we examined whether the white matter connectivity of the hippocampus to other brain regions was affected and related to memory decrements in participants with type 2 diabetes using fiber tractography based on diffusion MRI. In addition, local abnormalities in the hippocampal microstructure were investigated, but no differences were observed. We showed that participants with diabetes have reduced white matter connectivity between the hippocampus and the frontal lobe compared with participants without diabetes. For participants who scored lower on memory performance, independent of type 2 diabetes, tractography revealed reduced white matter connectivity of the hippocampus to the temporal lobe. In addition,

participants with diabetes scored lower on memory performance. These findings suggest that memory decrements in participants with type 2 diabetes appear to be associated with altered hippocampal white matter connectivity.

In **chapter 4**, we investigated whether the local microstructural and microvascular properties of the hippocampus were altered and related to the memory decrements in participants with type 2 diabetes using intravoxel incoherent motion imaging. Participants with type 2 diabetes showed increased hippocampal blood perfusion volume and blood flow. In addition, the increased blood flow was associated with lower memory performance, which was also observed in participants with both type 2 diabetes and lower memory scores. Furthermore, the local hippocampal microstructure was also altered in participants who scored lower on memory function. Our results suggests that, in addition to the hippocampal microstructure, especially the microvascular properties were altered in participants with both type 2 diabetes and memory problems, hinting at an underlying vascular mechanism which might act compensatory for these subtle cerebral alterations.

In **chapter 5**, we examined whether the blood supply from the common carotid artery to the brain and whether whole brain perfusion were altered and related to cognitive performance in type 2 diabetes by applying phase-contrast velocity-sensitive MR angiography and arterial spin labeling (ASL) MRI, respectively. We observed hypoperfusion, which was measured with ASL, in the subcortical gray matter of participants with type 2 diabetes, while no diabetes specific effects were observed for the carotid blood flow or cerebral blood flow in other cortical brain regions. In addition, no associations were observed in relation with cognitive function. These results suggest the involvement of an underlying vascular mechanism in diabetes, but whether the vascular mechanism also underlies the cognitive decrements remains inconclusive.

In **chapter 6**, it was investigated whether the neurotransmitters, i.e. the inhibitory γ-aminobutyric acid (GABA) and the excitatory glutamate, are related to type 2 diabetes and cognition. Higher GABA levels were found in participants with both type 2 diabetes and lower cognitive scores. No alterations in glutamate were observed in relation to diabetes and cognitive function. These results suggest the involvement of altered neurometabolite levels in the process of cognitive decrements in diabetes.

In **chapter 7**, using functional MRI, we examined whether the global functional networks were altered and related to information processing speed in participants with type 2 diabetes, pre-diabetes, and healthy controls. Participants with diabetes showed a more efficient organization of cerebral networks compared with healthy controls. In addition, participants with pre-diabetes showed intermediate results between the diabetes and control groups. Furthermore, lower processing speed was also associated

with a more efficient cerebral organization. These results hint at functional reorganization of the cerebral networks as compensatory mechanism for cognitive decrements.

Finally, in **chapter 8**, we discuss the main findings of the present thesis on the use of advanced MRI examinations in diabetes and their relation with cognitive performance in a broader perspective. In addition, their clinical implications, methodological considerations, and directions for future research are also addressed.

Nederlandstalige samenvatting

Diabetes mellitus, ook wel suikerziekte genoemd, is een chronische stofwisselingsziekte waarbij sprake is van herhaaldelijk verhoogde bloedsuikerwaarden. Deze worden veroorzaakt door onvoldoende of geen productie van het hormoon insuline en/of doordat het lichaam niet meer goed reageert op insuline. Diabetes kan onderverdeeld worden in verschillende typen, waarbij het overgrote deel (ongeveer 90%) van alle mensen met suikerziekte diabetes type 2 heeft en ongeveer 10% diabetes type 1. Diabetes type 1 komt vaak al voor in de kinderjaren en wordt veroorzaakt door te weinig of geen insuline productie. Diabetes type 2 ontwikkelt zich meestal op oudere leeftijd en wordt veroorzaakt doordat het lichaam minder goed reageert op insuline. Wereldwijd lijden miljoenen mensen aan diabetes (366 miljoen in 2011) en de verwachting is dat dit aantal zal toenemen naar 552 miljoen in 2030. In Nederland lijden ongeveer één miljoen mensen aan diabetes, waarvan een kwart niet eens weet dat ze deze aandoening hebben.

Op de lange termijn kan diabetes leiden tot microvasculaire en macrovasculaire complicaties. Onder microvasculaire complicaties worden onder andere aandoeningen aan de zenuwen, nieren en ogen verstaan door schade aan de kleine bloedvaten. Macrovasculaire complicaties worden veroorzaakt door schade aan de grote bloedvaten en verhogen het risico op hart- en vaatziekten zoals een hartinfarct, een herseninfarct en perifeer vaatlijden. In het verloop van hun ziekte ontwikkelen patiënten met diabetes type 2 vaak cognitieve problemen zoals geheugenproblemen en problemen met het verwerken van informatie. Daarnaast hebben deze patiënten op latere leeftijd een verhoogde kans op het ontwikkelen van dementie, waaronder de ziekte van Alzheimer. De exacte oorzaak van de cognitieve problemen is nog onbekend. Daarom is er meer inzicht nodig in de onderliggende processen die de cognitieve problemen in patiënten met type 2 diabetes veroorzaken. Met behulp van beeldvormende Magnetic Resonance Imaging (MRI) technieken is het mogelijk om de hersenen in beeld te brengen en processen in de hersenen te onderzoeken. Dit proefschrift richt zich met name op de meer geavanceerdere en niet-invasieve MRI technieken die eventuele subtiele afwijkingen vroegtijdig in de hersenen op kunnen sporen. Hopelijk leidt dit uiteindelijk tot de ontdekking van MRI kenmerken (biomarkers genaamd) die vroegtijdig hersenveranderingen kunnen opsporen, zodat die in de toekomst kunnen bijdragen aan het ontwikkelen van behandelstrategieën om de kans op het krijgen van cognitieve problemen en dementie, waaronder de ziekte van Alzheimer, te verkleinen. De vraag die daarom centraal staat in dit proefschrift is:

Kunnen geavanceerdere MRI technieken ons meer inzicht geven in de complexe relatie tussen diabetes type 2 en cognitieve problemen?

Een algemene introductie over diabetes en de relatie met cognitieve problemen en bevindingen met de MRI is beschreven in **hoofdstuk 1**. In dit hoofdstuk komen ook kort de twee onderzoeksopzetten aan bod die zijn gebruikt in dit proefschrift.

Hoofdstuk 2 geeft een literatuuroverzicht over de afwijkende bevindingen in de hersenen die gepaard gaan met zowel diabetes type 2 als de cognitieve problemen. In dit hoofdstuk komen verschillende MRI technieken aan bod die ofwel alleen klinisch gebruikt worden, ofwel alleen voor onderzoeksdoeleinden, ofwel voor beide. Deze MRI technieken onderzoeken verschillende aspecten van het brein en kunnen dus onafhankelijk van elkaar leiden tot andere of nieuwe inzichten. Zo zijn er MRI technieken die zich vooral richten op structurele onomkeerbare hersenafwijkingen (voornamelijk de standaard MRI technieken die klinisch gebruikt worden). De geavanceerdere MRI technieken focussen zich meer op de structurele en vasculaire eigenschappen van het brein op een zeer klein niveau (ook wel microstructurele en microvasculaire eigenschappen genoemd), op stofjes (metabolieten genaamd) in het brein, en op de hersenactiviteit. Uit dit hoofdstuk kunnen wij concluderen dat er verschillende processen, die allemaal invloed op elkaar hebben, ten grondslag liggen aan het ontstaan van cognitieve problemen in mensen met diabetes. Ook is duidelijk geworden dat de standaard MRI technieken, die voornamelijk klinisch worden gebruikt, al uitgebreid toegepast zijn in patiënten met diabetes, terwijl de meer geavanceerdere technieken tot nu toe weinig zijn toegepast. Het gebruik van deze geavanceerdere MRI technieken kan daarom in de toekomst een belangrijke rol spelen om een beter inzicht te krijgen in de complexe relatie tussen diabetes, hersenafwijkingen en de cognitieve problemen. In de volgende hoofdstukken van dit proefschrift worden de resultaten van de verschillende geavanceerdere MRI technieken besproken.

In **hoofdstuk 3** hebben wij een diffusie-gewogen MRI techniek toegepast. Met deze techniek kan de microstructurele oriëntatie van de witte stof (de zenuwbanen) in de hersenen bepaald worden. Door vervolgens 'fiber tractography' toe te passen kan worden berekend en gevisualiseerd welke gebieden structureel met elkaar verbonden zijn. Patiënten met diabetes type 2 hebben qua cognitie vooral problemen met het geheugen. Het gedeelte van het brein wat hier voornamelijk bij betrokken is, is de hippocampus. Daarom is in dit hoofdstuk onderzocht of de verbindingen (ook wel connectiviteit genoemd) tussen de hippocampus en andere hersengebieden zijn aangedaan en of deze gerelateerd kunnen worden aan de verminderde geheugen-prestaties van patiënten met diabetes. Daarnaast is ook naar de microstructurele eigenschappen van de hippocampus gekeken. De belangrijkste bevindingen laten zien dat deelnemers met diabetes een verminderde connectiviteit hebben tussen de hippocampus en de frontale kwab. Deze frontale kwab bevindt zich aan de voorkant van onze hersenen en is ook betrokken bij het geheugen. De microstructurele eigenschappen van de hippocampus bij deelnemers met diabetes type 2 bleken niet anders te zijn dan bij gezonde deelnemers. Verder werd geobserveerd dat deelnemers die lager scoorden op de geheugentesten, onafhankelijk of ze diabetes hebben, een verminderde connectiviteit hebben tussen de hippocampus en de slaapkwab (temporaal). Deze kwab ligt aan de zijkant (vlak boven de oren) van onze hersenen en

speelt ook een belangrijke rol met betrekking tot geheugen. Deze resultaten suggereren dat het achteruitgaan van het geheugen in deelnemers met diabetes type 2 mogelijk wordt veroorzaakt door een verminderde connectiviteit tussen de hippocampus en enkele hersenkwabben.

In **hoofdstuk 4** hebben wij de 'intravoxel incoherent motion' MRI techniek toegepast, een speciale diffusie-gewogen MRI techniek. Met deze techniek kan naast de lokale microstructurele eigenschappen tegelijkertijd ook naar de microvasculaire eigenschappen gekeken worden. Om dezelfde redenen als in hoofdstuk 3 is in dit hoofdstuk ook de hippocampus onderzocht op veranderingen met betrekking tot de lokale microstructurele en microvasculaire eigenschappen in relatie tot de achteruitgang van het geheugen. De belangrijkste resultaten in dit hoofdstuk zijn dat de microvasculaire eigenschappen in de hippocampus veranderd zijn in deelnemers met diabetes. Er werd een verhoogde doorbloeding van de hippocampus waargenomen bij deelnemers met diabetes type 2, namelijk een verhoogde bloedtoevoer en een toegenomen bloedvolume. De verhoogde bloedtoevoer in de hippocampus is geassocieerd met een lagere geheugenscore, onafhankelijk van het hebben van diabetes. Dezelfde associatie werd gevonden bij deelnemers die zowel diabetes als een lage geheugenscore hadden. Met deze MRI techniek bleek er ook een veranderde microstructuur in de hippocampus te bestaan bij mensen die lager scoorden op de geheugentesten, wat doet vermoeden dat de hippocampus beschadigd is. Er is bekend dat bij ernstige cognitieve problemen er een verlaagde doorbloeding van de hippocampus kan bestaan, terwijl wij juist een verhoogde doorbloeding vonden bij de deelnemende diabetici. Echter, de deelnemers met diabetes die hebben deelgenomen aan dit onderzoek, hadden relatief goede geheugenscores, nog behorend tot een normale geheugenfunctie. De verhoogde doorbloeding in de hippocampus bij de deelnemende diabetici kan daarom worden geduid als een compensatiemechanisme van de reeds beginnende hersenveranderingen door de diabetes, waarbij de geheugenscore dus nog normaal is.

In **hoofdstuk 5** zijn twee verschillende MRI technieken toegepast, namelijk een 'phase-contrast velocity-sensitive MR angiography' en een 'arterial spin labeling' MRI techniek. Met de eerstgenoemde MRI techniek is onderzocht of de bloedtoevoer in de halsslagader naar de hersenen verschilt tussen de deelnemers met en zonder diabetes. Met behulp van de tweede techniek hebben wij het brein onderzocht op verschillen in de doorbloeding van diverse hersengebieden. Ook is er gekeken of de uitkomstmaten van deze twee technieken gerelateerd konden worden aan lagere cognitiescores. Hiervoor hebben wij een algemene cognitiemaat berekend gebaseerd op drie verschillende testen. Deze drie testen waren gericht op geheugen, aandacht, flexibiliteit, snelheid, en hogere denkfuncties. De belangrijkste bevindingen laten zien dat de deelnemers met diabetes een verlaagde doorbloeding hebben in de subcorticale

gebieden. Deze gebieden liggen centraal en diep in de hersenen. Er werden geen verschillen gevonden in bloedtoevoer naar de hersenen toe en doorbloeding in andere gebieden tussen deelnemers met en zonder diabetes. Daarnaast werd er geen relatie gevonden tussen cognitieve prestatie en bloedtoevoer of doorbloeding. Deze resultaten tonen een vasculaire verandering bij diabetes, maar laten niet zien dat een vasculair mechanisme betrokken is bij de cognitieve achteruitgang die vaak gepaard gaat met diabetes.

In **hoofdstuk 6** hebben wij naar stofjes (metabolieten) in de achterhoofdskwab (occipitaal) van het brein gekeken met behulp van een MR spectroscopie techniek. De achterhoofdskwab ligt achterin het brein en is betrokken bij het verwerken van visuele informatie. In dit hoofdstuk hebben wij voornamelijk gericht op de twee belangrijkste neurotransmitters in het brein, namelijk glutamaat en GABA (ofwel voluit gamma-aminoboterzuur genaamd). Neurotransmitters zijn stofjes die signalen overdragen tussen de zenuwcellen (ook wel neuronen genoemd) en kunnen een stimulerende of een remmende werking op neuronen hebben. Glutamaat is één van de belangrijkste stimulerende neurotransmitters, terwijl GABA de belangrijkste remmende neurotransmitter in het brein is.

Wij hebben onderzocht of de concentraties van deze twee neurotransmitters in het brein veranderd waren in deelnemers met diabetes en of de neurotransmitter-concentraties gerelateerd konden worden aan cognitieve prestatie. Net zoals in hoofdstuk 5 hebben wij voor de cognitieve prestatie ook hier dezelfde algemene cognitiemaat gebruikt. De belangrijkste resultaten laten een verhoogde GABA concentratie zien in deelnemers die zowel diabetes hebben als minder cognitief presteerden. Er werd geen relatie tussen de concentratie glutamaat en diabetes of cognitieve prestatie gevonden. Andere stofjes in het brein lieten een lagere concentratie *n*-acetyl aspartate (NAA, een marker voor het normaal functioneren van de zenuwcellen) zien in deelnemers die minder cognitief presteerden, onafhankelijk van het hebben van diabetes. Ook werd er een verhoogde concentratie choline (onder andere betrokken bij het vervangen en repareren van de buitenwand (membraan) van een cel) gevonden in deelnemers die zowel diabetes hebben als minder cognitief presteerden. De belangrijkste conclusie met betrekking tot de neurotransmitters is dat deelnemers met diabetes die slechter scoorden op cognitie een veranderde GABA concentratie laten zien.

In **hoofdstuk 7** hebben wij de functionele MRI (fMRI) techniek toegepast. Met de fMRI techniek kan de hersenactiviteit, in termen van bloedoxygenatie (verhouding zuurstofrijk en zuurstofarm bloed), in de grijze stof van de hersenen gemeten worden. Door te kijken in welke mate het fMRI signaal tussen twee verschillende hersen-gebieden met elkaar samenhangt, kan berekend worden welke hersengebieden functioneel met elkaar zijn verbonden. Dit wordt ook wel functionele connectiviteit

genoemd. In dit hoofdstuk hebben wij een andere onderzoeksopzet gebruikt dan in de voorgaande hoofdstukken. Wij hebben naast gezonde deelnemers en deelnemers met diabetes ook deelnemers geïncludeerd die een groot risico lopen op het ontwikkelen van diabetes (ook wel prediabetes genoemd). Deze prediabetes deelnemers hebben verhoogde bloedsuikerwaarden ten opzichte van gezonde deelnemers, maar nog niet zulke hoge waarden in vergelijking met diabetes type 2. Daarnaast hebben deze prediabetes deelnemers ook twee of meer van de volgende kenmerken: 1) een te grote buikomvang, 2) verhoogd gehalte aan vetten in het bloed, 3) verlaagd HDL cholesterol (dit is het goede cholesterol dat het slechte cholesterol opruimt), en 4) een verhoogde bloeddruk. De aandoening van deze deelnemers wordt ook wel aangeduid met het metabool syndroom.

Wij hebben onderzocht of de functionele verbindingen tussen verschillende hersengebieden verspreid over het hele brein (ook wel functionele netwerken genoemd) zijn veranderd in deelnemers met diabetes type 2, prediabetes en gezonde deelnemers. De functionele netwerken zeggen iets over hoe goed verschillende gebieden met elkaar communiceren. Daarnaast is gekeken of de functionele netwerken gerelateerd konden worden aan de cognitieve prestatie van de deelnemers. Deze cognitieve prestatie was gebaseerd op de snelheid waarmee deelnemers informatie konden verwerken. De belangrijkste bevindingen laten zien dat deelnemers met diabetes beschikken over een beter functioneel netwerk in vergelijking met gezonde deelnemers. De prediabetes deelnemers beschikken ook over een beter functioneel netwerk ten opzichte van gezonde deelnemers, maar niet ten opzichte van deelnemers met diabetes. De resultaten van de prediabetes deelnemers liggen dus tussen de resultaten van de gezonde deelnemers en deelnemers met type 2 diabetes in. Daarnaast was een tragere snelheid van informatieverwerking geassocieerd met een beter functioneel netwerk, onafhankelijk van het hebben van diabetes. De betere functionele netwerken in deelnemers met diabetes type 2 en in mindere mate in deelnemers met prediabetes worden geduid als een compensatiemechanisme van de reeds beginnende veranderingen in de hersennetwerken door diabetes. Deze resultaten suggereren dat als deze compensatie faalt, dit tot uiting kan komen als merkbare achteruitgang in cognitieve functies. Dit moet echter nog nader onderzocht worden.

In het laatste hoofdstuk, **hoofdstuk 8**, worden de resultaten uit de voorgaande hoofdstukken geïntegreerd en in een breder perspectief geplaatst. Ook zijn de onderzoeksmethodes bediscussieerd, hebben wij de betekenis van de resultaten voor de kliniek besproken, en geven wij advies voor toekomstige studies die nodig zijn om de complexe relatie tussen diabetes type 2 en de cognitieve problemen verder te onderzoeken.

Uit dit hoofdstuk kunnen wij concluderen dat de geavanceerdere MRI technieken, toegepast in dit proefschrift, ons meer inzicht hebben gegeven in de complexe relatie

tussen diabetes type 2 en de cognitieve achteruitgang. Al voordat de cognitieve problemen of structurele onomkeerbare hersenafwijkingen (voornamelijk opgespoord met de standaard MRI technieken) klinisch zichtbaar worden, kunnen de geavanceerdere MRI technieken reeds vroegtijdig subtiele afwijkingen in de hersenen opsporen. Deze hersenafwijkingen zijn gerelateerd aan een subtiele achteruitgang in de cognitieve prestatie en worden voornamelijk veroorzaakt door vasculaire veranderingen in de hersenen en/of de verhoogde bloedsuikerwaarden. Deze twee oorzaken zullen onherroepelijk met elkaar in verbinding staan. De subtiele hersenveranderingen duiden op een compensatiemechanisme dat uiteindelijk, in een later stadium, onvoldoende zal zijn. Dit zal dan tot merkbare cognitieve problemen kunnen leiden. Het toepassen van deze geavanceerdere MRI technieken geeft dus meer inzicht in de onderliggende mechanismes die een rol spelen bij de cognitieve achteruitgang in diabetes. Deze MRI technieken zouden in de toekomst kunnen bijdragen aan het ontwikkelen en beoordelen van behandelstrategieën om de kans op het krijgen van cognitieve problemen en dementie te verkleinen.

Valorization addendum

The research work and its outcomes presented in this thesis have, besides scientific merit, also social and economic value. In this addendum we discuss the research approach and results into a broader perspective and indicate the valorization potential of the main findings.

Relevance of the study

Healthcare problem

Type 2 diabetes mellitus is associated with cognitive decrements, accelerated cognitive decline, and an increased risk for developing dementia and Alzheimer's disease. Dementia is an umbrella term of multiple neurodegenerative brain diseases, of which Alzheimer's disease is the most common type. Dementia causes a decline in mental processes, especially marked by decreases in memory function and changes in behavior. These signs may become severe enough to interfere with daily activities, leading to a decrease in the quality of life and depressive symptoms or a depression. This has a huge impact, both economically and emotionally, for the patient, the relatives of the patient, and imposes a burden on the healthcare system.

In 2015 already 46.8 million people worldwide suffered from dementia. It is expected that the number of individuals with dementia doubles every 20 years and will approximately reach to 74.7 million in 2030 and 131.5 million in 2050. This increase in the prevalence of dementia will be accompanied by an increase in costs attributed to (medical) healthcare by family and professional caregivers. These worldwide costs of dementia were already estimated in the US at $818 billion in 2015. In this regard, it is very important to identify early cerebral signs of cognitive decrements before the onset of dementia in order to identify patients at risk, for whom the cognitive decline can be halted, delayed or even prevented. In this regard, MRI could be beneficial, as advanced imaging techniques are able to investigate the condition of the brain and to identify subtle tissue alterations.

Investigational approach

In early prodromal stages of dementia, individuals will show slight decreases in memory function, which is one of the most commonly affected cognitive domains in type 2 diabetes. In the present thesis, multiple advanced MRI techniques were applied to identify cerebral biomarkers of cognitive decline in type 2 diabetes. These MRI biomarkers can provide information about the underlying mechanisms and tissue status, and hopefully allow in the future to the development of effective treatment for preventing or delaying cognitive decline and the development of dementia in type 2 diabetes or other individuals. Furthermore, the utility of the MRI biomarkers can make it possible to follow and evaluate the treatment process and, if needed, the obtained

results can be indicative for deciding to change the treatment. Therefore, it is important to investigate the condition of the brain tissue after each intervention with MRI, because alterations of brain tissue may perhaps explain or predict the cognitive decline in biological way.

Main findings

The present thesis identified subtle cerebral alterations in type 2 diabetes who also scored lower on cognitive function than control participants without diabetes. These findings could represent early signs of tissue changes related to cognitive decrements, dementia, and/or Alzheimer's disease. In brief, altered metabolite levels (occipital lobe) were observed which were linked to the involvement of an underlying glucose mechanism. Furthermore, altered microvasculature was observed in the hippocampus hinting at an underlying vascular mechanism, which might act compensatory to subtle cerebral alterations. In addition, the involvement of an underlying neurovascular coupling mechanism, which might be a compensatory mechanism as well, was shown in participants with type 2 diabetes and pre-diabetes, who revealed a more efficient cerebral organization compared with healthy participants. This effect might be attributed to cerebral adaptation to counteract the slight decrements in cognitive performance. Moreover, participants with type 2 diabetes also showed reduced connectivity between the hippocampus and the frontal lobe, and hypoperfusion in the subcortical gray matter region. Efforts to improve the vasculature and to control the glycemic blood levels might have a significant implication to prevent or treat the development of cognitive decline. These MRI biomarkers could play an important role to evaluate interventions or identifying high-risk patients, but further research is warranted to validate these biomarkers. This addendum therefore includes a proposal for further studies.

Target population

The results of the present thesis are important for individuals with diabetes and individuals who are at a high risk to develop diabetes. Individuals should become aware that diabetes is a risk factor to develop cognitive problems, dementia, and Alzheimer's disease. Therefore, prevention or a better control of diabetes (i.e. well-controlled blood glucose levels) should have more attention. Besides a better control of the blood glucose levels, paying attention to a healthier lifestyle also could have a beneficial effect on diabetes and the brain.

Another target group for whom the results of the present thesis can be interesting are pharmacists and the pharmaceutical industry. As the results showed that slight decreases in cognitive performance were reflected by subtle cerebral alterations in

type 2 diabetes, even before the onset of clinically manifest cognitive problems, the development of new medication to delay or even prevent the worsening of cognition should be encouraged. In particular, pharmacists and the pharmaceutical industry should focus on medication to control the blood glucose levels, metabolic profile, and to improve the vascular function, because these mechanisms, which are inextricably linked to each other, seem to be involved in the process of cognitive decrements in type 2 diabetes. The advanced MRI techniques applied in the present thesis could then be used as a tool to evaluate the condition of the brain after administering the newly developed medication. In addition, as mentioned previously, these MRI techniques could also be used to evaluate the effect of lifestyle changes (i.e. weight loss, physical exercise) on the brain.

Innovation and future

The application of advanced and noninvasive MRI techniques used in the present thesis is an innovative approach to identify neuronal MRI correlates of cognitive decrements in type 2 diabetes. These MRI techniques provide more insight into the pathophysiology at the microstructural, microvascular, metabolic, and functional level. The new results showed that the advanced MRI techniques were able to identify (potentially) early cerebral biomarkers of cognitive decrements in type 2 diabetes, as the observed cognitive decrements were rather small. The first results of the present thesis are promising. However, more research and ideally a longitudinal setup are needed to validate whether the measured effects have the potential to serve as an early biomarker to identify individuals who are at high risk to develop dementia. Treatment of early signs may be more effective than for rather late signs. This may also have an enormous impact on reducing the healthcare costs when future and longitudinal studies are able to identify high-risk individuals.

From a clinical point of view, controlling or treating the blood glucose levels, metabolic levels, and to improve the vascular function may reduce the cognitive decrements and hopefully reduce the risk to develop dementia. Future clinical studies could use these advanced MRI techniques and the acquired biomarkers to evaluate the effect of newly developed treatment or intervention strategies on the brain. When the new medications are found to be beneficial for patients, it will eventually lead to an economically benefit for the healthcare system through the reduced incidence of dementia. Even more important, it may lead to an increase of the quality of life for the patient (and their caregivers/relatives) by having less cognitive constraints and depressive symptoms, which are often associated with each other.

Dankwoord

Dankwoord

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Curriculum vitae

Frank Cornelius Gerardus van Bussel was born on November 5th 1985 in Deurne, the Netherlands. After graduating secondary school in 2005 at the St.-Willibrord Gymnasium in Deurne, the Netherlands, he started his academic education at Eindhoven University of Technology, where he obtained his Bachelor of Science degree (BSc) in August 2009 and his Master of Science degree (MSc) in Medical Engineering in August 2011. During the Master Program, he did an internship at the Vascular Bioengineering Laboratory of the McGowan Institute for Regenerative Medicine, University of Pittsburgh, PA, USA (October 2009 – January 2010), under the supervision of prof. dr. David A. Vorp and dr. David E. Schmidt on the topic: "Reconstruction of patient specific Abdominal Aortic Aneurysm 3D models involving iliac and renal bifurcations from CT images". Furthermore, he did his final Master project, entitled "Effect of transient high blood glucose on brachial artery flow mediated dilation, distensibility and plasma biomarkers of endothelial function", at the department of BioMedical Engineering at the Cardiovascular Research Institute Maastricht (CARIM) of Maastricht University under the supervision of dr. ir. Koen D. Reesink and prof. dr. ir. Arnold P. Hoeks. In September 2011 he started his PhD project at the department of Radiology at the School for Mental Health and Neuroscience (MHeNs) of Maastricht University Medical Center. The research, as described in the present thesis, was performed under the supervision of promotores prof. dr. ir. Walter H. Backes and prof. dr. Paul A.M. Hofman, and copromotor dr. Jacobus F.A. Jansen. During his PhD project, Frank's work was awarded with student stipends and presentations at multiple international scientific conferences.

List of publications

Thesis

van Bussel FC, Backes WH, Hofman PA, van Oostenbrugge RJ, van Boxtel MP, Verhey FR, Steinbusch HW, Schram MT, Stehouwer CD, Wildberger JE, Jansen JF: Cerebral pathology and cognition in diabetes: The merits of multiparametric neuroimaging. *Submitted*

van Bussel FC, Backes WH, Hofman PA, van Boxtel MP, Schram MT, Stehouwer CD, Wildberger JE, Jansen JF: Altered hippocampal white matter connectivity in type 2 diabetes mellitus and memory decrements. *Journal of Neuroendocrinology 2016;28. doi: 10.1111/jne.12366*

van Bussel FC, Backes WH, Hofman PA, van Oostenbrugge RJ, Kessels AG, van Boxtel MP, Schram MT, Stehouwer CD, Wildberger JE, Jansen JF: On the interplay of microvasculature, parenchyma, and memory in type 2 diabetes. *Diabetes Care 2015;38:876-882. doi: 10.2337/dc14-2043*

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van Bussel FC, Backes WH, Hofman PA, Puts NA, Edden RA, van Boxtel MP, Schram MT, Stehouwer CD, Wildberger JE, Jansen JF: Altered GABA concentrations in type 2 diabetes mellitus are related to lower cognitive functioning. *Submitted*

van Bussel FC, Backes WH, van Veenendaal TM, Hofman PA, van Boxtel MP, Schram MT, Sep SJ, Dagnelie PC, Schaper N, Stehouwer CD, Wildberger JE, Jansen JF: Functional brain networks are altered in type 2 diabetes and pre-diabetes: Signs for compensation of cognitive decrements? - The Maastricht Study -. *Submitted*

Other

van Bussel FC*, van Bussel BC*, Hoeks AP, Op 't Roodt J, Henry RM, Ferreira I, Vanmolkot FH, Schalkwijk CG, Stehouwer CD, Reesink KD: A control systems approach to quantify wall shear stress normalization by flow-mediated dilation in the brachial artery. *PloS one* 2015; 10(2): e0115977.

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Moonen AJH, Dujardin K, Behal H, Defebvre L, Duhamel A, Duits AA, Plomhause L, Tard C, Hofman PA, **van Bussel FC**, Leentjens AFG: Reduced grey matter density associated with early cognitive decline in Parkinson's disease: a voxel-based morphometry study. *Submitted*

* both authors contributed equally

Oral presentations

7th Annual Meeting of the International Society for Magnetic Resonance in Medicine Benelux Chapter, January 16th, Ghent, Belgium, 2015

Joint Annual Meeting of the International Society for Magnetic Resonance in Medicine – European Society for Magnetic Resonance in Medicine and Biology 2014, Section for Magnetic Resonance Technologists 23rd Annual Meeting, May 10-16, Milan, Italy, 2014

6th Annual Meeting of the International Society for Magnetic Resonance in Medicine Benelux Chapter, January 20th, Maastricht, the Netherlands, 2014

1st scientific meeting of The Maastricht Study, April 12th, Maastricht, the Netherlands, 2013

Poster presentations

23rd Annual Meeting & Exhibition of the International Society for Magnetic Resonance in Medicine, May 30 - June 5, Toronto, Ontario, Canada, 2015

Joint Annual Meeting of the International Society for Magnetic Resonance in Medicine – European Society for Magnetic Resonance in Medicine and Biology 2014, Section for Magnetic Resonance Technologists 23rd Annual Meeting, May 10-16, Milan, Italy, 2014

21st Annual Meeting & Exhibition of the International Society for Magnetic Resonance in Medicine, April 20-26, Salt Lake City, Utah, USA, 2013

5th Annual Meeting of the International Society for Magnetic Resonance in Medicine Benelux Chapter, January 14th, Rotterdam, the Netherlands, 2013

16th Euron PhD meeting, September 27-28, Maastricht, the Netherlands, 2012